User Guide to the SPOT Insight Camera

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About This Manual

Introduction

The manual contains instructions for the SPOT Insight cameras (Color and B/W) and version 3.5 of the accompanying SPOT software. An on-line version of this manual in PDF (portable document format) is also included on the CD that contains the SPOT software. Refer to the **Installation Guidelines** chapter for more information on accessing and using the on-line manual.

Document Conventions

The following is a list of the textual conventions used in this manual:

Notes

Note: This is an example of a note. Notes appear within the text to indicate information for further consideration or reference.

Cautions

CAUTION!

This is an example of a cautionary note. Cautionary notes appear to indicate that you should carefully consider the implications of an action.

Warnings

WARNING

THIS IS AN EXAMPLE OF A WARNING. WARNINGS INDICATE THAT THE ACTION YOU ARE TAKING COULD EITHER CAUSE INJURY TO YOURSELF OR COULD HARM YOUR SYSTEM

Insight B/W

Insight B/W Insight B/W notes indicate specific instructions for users with the monochrome version of the SPOT Insight camera. In most cases, these notes indicate that a color related option is unavailable.

Software Development

Software Development informational notes indicate special circumstances related to the current release of the SPOT software. These notes also describe how to obtain free updates from Diagnostic Instruments.

Organization of the Manual

The *User Guide to the SPOT Insight Camera* is structured as shown in the table below. In addition to the chapter names and descriptions, the table indicates whether the chapter applies to the Basic program mode, the Advanced program mode, or both:

Chapter	Description	Basic or Advanced
About This Manual	Describes document conventions, manual structure.	Both
Installation Guidelines	Describes hardware/software installation.	Both
Quick Start	Steps for immediately capturing your first image in the Basic Program Mode.	Basic
Ch. 1 – New Features	Describes the software enhancements for version 3.3 (Windows and Mac editions) of the SPOT software.	Both
Ch.2 – Basic and Advanced Program Modes	Compares the features of the Basic and Advanced program modes.	Both
Ch. 3 – The Basic Program Mode	Details the SPOT Basic program mode, which is designed for beginning users who want to start taking pictures immediately without having to make a lot of processing decisions.	Basic
Ch. 4 – The Advanced Program Mode	Describes the SPOT Advanced program environment, including basic file commands and preliminary setup instructions.	Advanced
Ch. 5 – Preparing to Take a Picture	Describes the different procedures involved in setting up the software for image captures.	Advanced
Ch. 6 – Using Live Images	Describes SPOT's real-time imaging functionality.	Advanced
Ch. 7 – Taking a Picture	Describes the image capture process.	Advanced
Ch. 8 – Viewing, Editing, and Annotating Images	Describes the rich set of image editing and customization features in the SPOT software.	Advanced
Ch. 9 – Using SPOT Databases	Describes the procedures for creating and working with SPOT image databases.	Advanced
This functionality is not yet available for Mac users. Refer to the Free Software Updates *section below for details on obtaining updated versions of the software.*		
Ch. 10 – Reporting	Describes the Advanced mode reporting functionality in the SPOT software.	Advanced
This functionality is not yet available for Mac users. Refer to the Free Software Updates *section below for details on obtaining updated versions of the software.*		
Ch. 11 – Printing Images	Describes the procedures for printing images from the SPOT program.	Both
Ch. 12 – Using Other Windows Imaging Applications in Advanced Mode	Describes how to capture images (via an Advanced Mode TWAIN interface) with the SPOT camera using other Windows-based imaging programs such as Photoshop or Image-Pro Plus.	Advanced

(cont.)

Chapter	Description	Basic or Advanced
Ch. 13 – Using Other Windows Imaging Applications in Basic Mode	Describes how to capture images (via a Basic Mode TWAIN interface) with the SPOT camera using other Windows-based imaging programs such as Photoshop or Image-Pro Plus.	Basic
Ch. 14– Using Other Mac Imaging Applications	Describes how to capture images with the SPOT camera using other Mac imaging programs such as Photoshop or Image-Pro Plus.	Advanced
Ch. 15– Using Other Mac Imaging Applications in Basic Mode	Describes how to capture images (via a Basic Mode 3rd party interface) with the SPOT camera using other Mac imaging programs such as Photoshop or Image-Pro Plus.	Basic
Appendix A: Electronic Imaging Theory and SPOT Cameras	An introduction to the theory behind SPOT cameras.	Both
Appendix B: Specifications	Technical specifications for the SPOT hardware and software.	Both
Appendix C: Keyboard and Toolbar Button Commands	A table of menu commands and their keystroke and button equivalents.	Both
Appendix D: Selected Error Messages	A table of errors, causes, and solutions	Both
Appendix E: Converting Previous Version SPOT Databases	Instructions for performing a one-time conversion of SPOT databases from earlier versions of the software.	Advanced
Appendix F: Third Party Software	A list of imaging software systems and their levels of compatibility with SPOT, RT, and Insight cameras.	Both

Free Software Updates

Registered SPOT camera owners can download free software updates via the Internet. When you register your product, Diagnostic Instruments supplies you with the user name and password needed to download the latest release of the software, as well as PDF user guides, software developer kits, and SPOT third party DLLs. To register, contact Diagnostic Instruments at 1 (586) 731-6000. You should periodically check Diagnostic Instruments' web site **(www.diaginc.com/software)** for the latest release.

To download files, follow these steps:

1. Access the software download page on Diagnostics Instruments' web site – **www.diaginc.com/software**.

2. Click on the link for your software/operating system. You are prompted for a user name and password.

3. Enter your **User Name** and **Password**. If you have not yet registered your product, contact Diagnostic Instruments at 1 (586) 731-6000 to do so and obtain the user name and password.

4. Click **OK**.

Installation Guidelines

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QUICK REFERENCE:

- Compare the minimal and optimal hardware requirements for using the SPOT Insight camera... pp. x – xiv
- Set up an Epson printer for use with the SPOT software... p. xiv
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Introduction

The following sections detail the system and installation guidelines for using the SPOT Insight camera and v 3.5 of the SPOT software. They are as follows:

- System Requirements
- What is in the Box
- Installing the Software
- Installing the Hardware
- Using SPOT's On-line Documentation

System Requirements

The following sections details the hardware and software requirements needed for the SPOT Insight camera/software. Minimum and optimal system recommendations are detailed for each item.

Equipment Standards - Windows

	Minimum	Optimal System Recommendations
Processor	Pentium, 166 MHz	***Dell Dimension 4100*** with 1 GHz Pentium III Processor In addition to the fact that this is a fast and reliable machine, there are many good reasons to order from Dell, including: • Easy to use web site • On-line ordering and product selection • One week shipping time • Excellent technical support
Video Card	A card that supports 24 bit, true color at the desired resolution with a refresh rate above 70 Hz.	***32 Mb Nvidia TNT2 M64 AGP Graphics Card*** This is the standard card offered on the aforementioned Dell Dimension 4100. For customers who want to videotape their live image output, Diagnostic Instruments recommends the following cards: • **Matrox G400 Dual Head video card** • **ATI Radeon 64 Mb VIVO card** Both of these cards have video outputs for connection to a standard VCR. All of the above cards offer refresh rates of 85 MHz or more at 1600 x 1200 resolution. All of these cards provide displays with sharp resolution, superb color quality, and clean, artifact-free images.
RAM	64 Mb	***256 Mb*** of RAM is recommended for optimal smoothness of operation.
PCI Slot		

With some video cards, you might experience system crashes or display problems in live image mode; you might also experience display problems when zooming in on captured images. To correct these problems, lower the Graphics Acceleration setting in Windows, as described in the "Video Card Related Problems" section below.

Equipment Standards – Windows (cont.)

	Minimum	Optimal System Recommendations
Monitor	Any color monitor with at least 800 x 600 pixel resolution at 24 bit RGB, and a refresh rate over 70 Hz.	***Hitachi CM 815 Plus*** This is Hitachi's latest 21" monitor, the replacement for the 814. It has perfect focus from the center to the corners of the screen, very fine resolution, and consistent color rendition. A very bright monitor, the 815 can display brilliant images even in high ambient light environments. It is also compact by comparison to other 21" monitors.
Operating System	Windows 95/98/NT/ 2000/ME/XP	***Windows XP*** Microsoft intends to replace all versions of the Windows operating system with Windows XP. Previous versions of the Spot software experienced significantly slower Live image frame rates when operating under Windows NT/2000 than when operating under Windows 95/98/ME. This situation has been rectified. With v3.5 of the Spot software, live image frame rates are equally fast with ALL versions of Windows, including XP.
Printer		***Epson Stylus Photo 750*** This printer produces high resolution, true color, 8.5 x 11 glossy prints that look better than those produced by many dye sub printers. It is perfect for printing reports that contain a mixture of images and text. It sells for an incredible $298. Refer to the "Epson Printer Settings" section below for details on how to optimize print quality.

Additional Hardware and Software

Web Browser

Internet Explorer 4.0 or greater.

This requirement applies only to Windows 95 users. Current releases of Internet Explorer are available as free downloads from **www.microsoft.com/downloads.**

Coupler

A digital camera coupler designed to fit a camera with a C mount.

Diagnostic Instruments makes couplers for most microscopes. They are available from the dealer where the camera was purchased. Diagnostic Instruments suggest the **D10XXC – 1x C-Mount Coupler**, which works with most microscopes. If the D10 series will not work with your microscope, Diagnostic Instruments suggests the following alternatives:

- **HRU060-CMT coupler (0.60x)** and the appropriate bottom clamp.
- **HRD100-CMT coupler (1x)** and the appropriate bottom clamp.

Lens

C- Mount lens for 1" format CCD

Light Source

Because the SPOT Insight camera is capable of extremely short exposure times, light sources whose intensity varies with the waveform of the 60 Hz line supply, should be avoided. Problem light sources include fluorescent lamps, 120 volt incandescent bulbs, and 6 volt 20 watt halogen lamps. These light sources cause the Live Image window to flicker and result in incorrect brightness and color balance readings in captured images. We have found that the low voltage halogen/xenon lamps used on most microscopes work well, as do the mercury burners used on fluorescence illuminators.

If your work requires you to use a light source that is problematic, you can use the Minimum Exposure option on the Image Setup window to avoid flicker and give you correct brightness and color balance readings. Refer to Ch. 5 – Preparing to Take a Picture for more information.

Equipment Standards - Mac

	Minimum	Optimal System Recommendations
Processor	PowerPC computer with a PCI bus	***G4 with a Hitachi CM815 Plus monitor*** (Note that the Apple 21" Studio Display has been discontinued) Note that the G4 and "Blue and White" G3 computers have new 64 bit PCI bus connectors that are larger than the standard 32 bit PCI edge card connector used on the SPOT PCI bus plug-in card. Despite the apparent size discrepancy, the SPOT cards work when plugged in – the small slot on the SPOT edge connector lines up with the bar on the PCI bus connector.
	Note: Apple Power Macintosh models 6100, 7100, and 8100 are PowerPCs, but they do not have a PCI bus, and thus cannot be used with the SPOT camera. The iMac does not have a PCI bus and cannot be used.	
Video Memory/ Card	Enough video memory on either the mother board or a plug in video card to display millions of colors at the desired resolution, and with a refresh rate above 70 Hz	The ***ATI Rage 128*** plug-in card that is standard on G4 and "Blue and White" G3 computers works well. The ***ATI Radeon*** card is recommended for 19" and larger monitors used with Macs other than the G4 and "Blue and White" G3 models.
RAM	64 Mb	***256 Mb*** of RAM is recommended for optimal smoothness of operation.
PCI Slot		
Monitor	Any color monitor with at least 800 x 600 pixel resolution at 24 bit RGB, and a refresh rate over 70 Hz.	***Hitachi CM 815 Plus*** This is Hitachi's latest 21" monitor, the replacement for the 814. It has perfect focus from the center to the corners of the screen, very fine resolution, and consistent color rendition. A very bright monitor, the 815 can display brilliant images even in high ambient light environments. It is also compact by comparison to other 21" monitors. Note that the 21" Apple Studio Display has been discontinued.
Operating System	Mac OS 8.6 through OSX	
Printer		***Epson Stylus Photo 750*** This printer produces high resolution, true color, 8.5 x 11 glossy prints that look better than those produced by many dye sub printers. It is perfect for printing reports that contain a mixture of images and text. It sells for an incredible $298.

Equipment Standards – Mac (cont.)

Additional Hardware and Software

Image Editing Software (optional)

The SPOT 3rd Party Interface, which is included with Mac software, is a stand-alone mini-application designed for Mac users who want to use the SPOT camera to capture images, and then edit the images in another imaging application. Some commonly used third party applications include:

- Adobe Photoshop (v. 3.0 or higher)
- Image Pro Plus (v. 3.0 or higher)
- Color It (v. 3.2 or higher)

The image editing software used with the SPOT 3rd Party Interface does not need to be TWAIN compliant. Refer to Ch. 13 – Using Other Mac Imaging Applications for more information.

Coupler

A digital camera coupler designed to fit a camera with a C mount.

Diagnostic Instruments makes couplers for most microscopes. They are available from the dealer where the camera was purchased. Diagnostic Instruments suggest the **D10XXC – 1x C-Mount Coupler**, which works with most microscopes. If the D10 series will not work with your microscope, Diagnostic Instruments suggests the following alternatives:

- **HRU060-CMT coupler (0.60x)** and the appropriate bottom clamp.
- **HRD100-CMT coupler (1x)** and the appropriate bottom clamp.

Lens

C- Mount lens for 1" format CCD

Light Source

Because the SPOT Insight camera is capable of extremely short exposure times, light sources whose intensity varies with the waveform of the 60 Hz line supply should be avoided. Problem light sources include fluorescent lamps, 120 volt incandescent bulbs, and 6 volt 20 watt halogen lamps. These light sources cause the Live Image window to flicker and result in incorrect brightness and color balance readings in captured images. We have found that the low voltage halogen/xenon lamps used on most microscopes work well, as do the mercury burners used on fluorescence illuminators.

If your work requires you to use a light source that is problematic, you can use the Minimum Exposure option on the Image Setup window to avoid flicker and give you correct brightness and color balance readings. Refer to Ch. 5 – Preparing to Take a Picture for more information.

Epson Printer Settings (Windows)

To optimize print quality when printing images with an Epson Photo Stylus 750 printer on a PC running Windows 98, do the following:

- Use photo quality, glossy paper.
- Set the Windows printer preferences as follows (after you have installed the printer):
  a) From the Start menu, select **Settings > Printers**. The Printers window displays all of the printers that are available to you.
  b) Right click on the Epson Stylus Photo 750 icon, and select the **Properties** option. The properties window for the printer appears.
  c) Click on the **Main** tab. The properties window should now appear as shown at right.
  d) Set the **Mode** to **Advanced** (i.e., click on the Advanced button).
  e) Click on **More Settings**, and set the following parameters:
     - Print Quality – ***Super Fine 1440 dpi***
     - Media Type – ***Photo Paper***
     - Ink – ***Color***
     - MicroWeave – Ensure that Super is ***not*** checked.
     - Flip Horizontal – Ensure that this option is ***not*** checked.
     - Color - ***ICM***
  f) Click on OK. The **Current Settings** under the **Main** tab on the properties window should appear as you defined them.
  g) Click on **Apply** and then click on **OK** to close the window.

What is in the Box

In addition to the manual, the shipping box should contain the following items:

- Camera head with a dust cap
- Power supply (with cable to camera)
- Power cord for the power supply
- PCI interface board
- 50 pin data cable to connect the interface board to the camera (10 feet long)
- Grounding wrist strap
- One CD-R that contains:
  - The software required to operate the SPOT camera.
  - A PDF (portable document format) version of the user manual, User Guide to the SPOT Insight Camera.
  - The Adobe Acrobat Reader software that is required to read and/or print the user's manual. Refer to "Using SPOT's On-line Documentation" below for instructions on installing the Reader on your computer.

- A registration card
- 1 year limited warranty

Installing the Software

The CD-R disk provided with your camera contains the SPOT software, which can be used with:

- Windows 95/98/NT/2000/ME/XP
- Mac OS 8.6 or higher

Note: Although the SPOT software can be installed either before or after installing the PCI card, Diagnostic Instruments recommends installing the software first.

Note: In some instances, Diagnostic Instruments has found that SPOT users with the **Windows 2000** *operating system will get a* **No response from camera...** *message when trying to either capture an image or view a live image. This is due to incorrect BIOS settings. For information on correcting this problem, refer to* Appendix D: Selected Error Messages.

Windows Installation

1. Ensure that all other application programs are closed. Windows NT and Windows 2000 Users should also ensure that they are logged on with administrator privileges.
2. If it is open, close the MS Office 95/97/2000 Tool Bar.
3. Insert the disk into your CD drive. The SPOT InstallShield Wizard automatically runs, and the Setup window appears.
4. Click on **Next** at the Readme Information window. The **User Information** window appears. Enter your name and company name.
5. Click on **Next**. The **Choose Destination Location** window appears. Either press the Browse button to specify the destination directory, or click on Next to accept the default directory, **C:\SpotCam**.
6. The **Select Program Folder** window appears. Click on **Next**. The Start Copying Files window displays the settings that you selected. Either click on Next to accept the settings, or click on Back to return to the previous screens and revise the settings.
7. After you have accepted the settings, the program files are copied over, and the installation program prompts you with the message **Will there be a SPOT camera connected this computer?**
8. Select **Yes**. The installation program runs.
9. When the installation is complete, shut down your computer, and install the plug-in card, as described in the **Installing the Hardware** section of this chapter. At this point, if you plan to use multiple SPOT cameras with the computer, you can install all of the PCI cards.
10. Re-start your computer. You should see the following two desktop shortcuts.

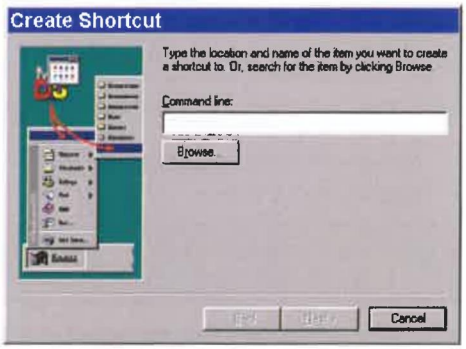
Use the SPOT Advanced program mode if you are an experienced user, or you are a new user who wants to explore the broad range of functionality and choices offered by the program.

Use the SPOT Basic program mode if you are a new user who wants to immediately begin taking high quality pictures without having to make a lot of processing decisions.

To replace a shortcut that was deleted from the desktop, follow these steps:

a) Right click on the desktop.

b) Select **New > Shortcut**. The Create Shortcut window appears.


Create Shortcut
Type the location and name of the item you want to create a shortcut to. Or, search for the item by clicking Browse.
Command line:
Browse...
Cancel

c) Enter the information in the **Command line** box:

- For the **Advanced** program, enter **C:\Spotcam\Spot32.exe -a**
- For the **Basic** program, enter **C:\Spotcam\Spot32.exe**.

If you found the file by browsing, you will see only one **Spot32.exe** program. If the shortcut is for the basic program, you will have to modify the command line, as described above.

d) Click on **Next**.

e) Name the shortcut.

f) Click on **Finish**. The new shortcut appears on the desktop.

If you installed PCI cards for more than one camera, you will be prompted to select a camera when you open the SPOT program.

Video Card Related Problems (Windows)

Diagnostic Instruments has found that the certain video cards can cause the SPOT program to crash when using the Live Image feature. If you experience this problem, change the Graphics Acceleration in Windows to a lower setting. Follow these steps:

1. From the **Start** menu button, select **Settings > Control Panel**.
2. Double click on the **Display** icon,
3. Click on the **Settings** tab.
4. Click on the **Advanced** button.
5. Click on the **Performance** tab.
6. Change the **Hardware Acceleration** setting by moving the slider to a lower setting. With some cards, you might have to move the slider to the lowest setting in order for live imaging to work.
7. Click on **Apply**.
8. Click on **OK**.
9. Click on **Close**.
10. Restart your computer before using the SPOT program.

Windows 95 Related Errors

If, after installing the PCI board you cannot access Windows 95, or if the camera will not communicate with the PC, one of the following situations might be the cause:

- The slot that you selected for the SPOT PCI board might have been occupied (previously) by a board from another device.
- The PCI slot s in the shared position (the PCI slot immediately adjacent to an ISA slot).

These are areas that Diagnostics Instruments has found to be problematic on *some* installations. This is due to the fact that Windows 95 is not fully plug and play compliant. To resolve the problem, try plugging the board into another PCI slot.

Windows NT Related Errors

If, when using Windows NT, your computer freezes during an image capture process (i.e., Get Image, Live Image viewing. Compute Exposure, etc…), it is may be due to a problem with a setting in your computer's BIOS. To correct this problem, follow these steps:

1. Access the BIOS settings. The method for accessing BIOS settings varies from computer to computer. If you are unsure of how to do this, contact your MIS department or an IT professional.
2. Go to the **Advanced Setup** area. This is the typical location, but it can vary according to the make of your computer.
3. Access the **PCI Bus Mastering** setting. The heading that you will see depends on the make of your computer.
4. Change the setting to **ENABLED.**
5. Restart the computer.

WARNING

DO NOT MODIFY OTHER BIOS SETTINGS. ALTERING OTHER SETTINGS COULD CAUSE SYSTEM INSTABILITY OR RENDER THE SYSTEM UNUSABLE.

Mac Installation

The CD-R disk provided with your camera contains the SPOT software, which can be used with Mac OS 8.6 or higher. ***If you are installing the SPOT software on a system with* Mac OSX, *ensure that you have an administrator password* BEFORE BEGINNING THE INSTALLATION.**

To install the SPOT software on a Mac, follow the steps listed below.

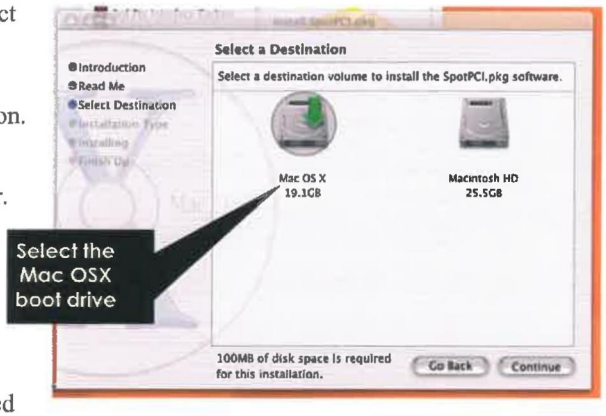
1. Insert the disk into your CD drive. The SPOT window appears.
2. Double click on the installer icon.
3. Click on **Continue**. The Spot 3.5 Installer window appears.
4. Select the installation location for the program.
5. Click on **Install**. The Installing progress indicator appears. When the installation is complete, you will be prompted with one of two messages, depending on the version of the Mac OS that you are using:
   - ***If you are using Mac OS 8.6 – 9.x***, the installation is complete. The program should indicate that the installation was successful. Quit the installer program, and restart the computer.
   - ***If you are using Mac OSX***, the **Apple Package Installer** program, which installs the device driver for the SPOT camera, launches and immediately prompts you to enter an **Administrator** password, as shown at right.

Authorization
Introduction
You need an Administrator password to install the software.
Click here to enter an Administrator password
Click the lock to make changes.
Close

WARNING

DO NOT CLICK ON THE CLOSE BUTTON. IF YOU DO, THE DEVICE DRIVER WILL NOT INSTALL, AND YOU WILL NOT BE ABLE TO OPERATE THE CAMERA.

6. Enter an administrator password. To enter the password, click on the lock icon. ("**Click on the lock to make changes**"). After you have entered a password, the SPOTPCI.pkg welcome screen appears.
7. Click on **Continue**. An informational screen appears.
8. Click on **Continue**. The program prompts you to select an installation destination, as shown at right.
9. Select the **Mac OSX** boot drive as the installation destination. The driver must be installed in this location.
10. Click on **Continue**.
11. When the installation is complete, restart the computer.



Mac OS 8.6 - 9.0 Installation Problems

If you are having difficulty installing the SPOT software with OS 8.6 – 9.0 systems, you will need to download and install the latest CarbonLib Apple Extension. This is a standard extension included with the Mac OS, and is offered as a free download/upgrade from the Apple support website – www.apple.com/support. After upgrading your CarbonLib extension you should be able to install the SPOT software.

Installing the Hardware

There are two main steps to installing the hardware:

- Installing the PCI board
- Connecting the camera to the microscope

Note: Although the SPOT software can be installed either before or after installing the PCI card, Diagnostic Instruments recommends installing the software first.

WARNING

This (the SPOT camera) is a Class A Product. In a domestic environment this product may cause radio interference in which case the user may be required to take adequate measures.

Installing the PCI Board

To install the PCI board, follow these steps:

CAUTION!

If you are unfamiliar with the procedures for installing plug in boards, refer the installation to your MIS department or to a qualified computer technician.

1. Turn off the computer, and let it sit for a few minutes to let the power supply drain.
2. Unplug all cables, and remove the power cord from the back of the computer.

WARNING

TO AVOID ELECTRICAL SHOCK, ALWAYS REMOVE THE POWER CORD AND ALL EXTERNAL CABLES, AND WAIT A FEW MINUTES FOR THE POWER SUPPLY TO DRAIN BEFORE OPENING THE CASE.

3. Place the computer on a clean, uncluttered surface, and remove the cover.
4. Position the computer so that the mother board is facing you.

WARNING

DO NOT HANDLE THE PCI INTERFACE BOARD OR TOUCH COMPONENTS INSIDE THE COMPUTER WITHOUT USING THE ENCLOSED GROUNDING WRIST STRAP.

5. To discharge any static charge that might have built up on your body, touch your finger to the metal case of the computer's power supply.
6. Stick the copper foil end of the grounding wrist strap to the metal case of the computer's power supply.
7. Loop the other end of the strap around your wrist by pressing the sticky side against the strap, keeping the dark strip against your skin.
8. Use your computer's manual to identify the location of the PCI slot(s) on the motherboard.
9. Remove the metal cover plate adjacent to an available PCI slot. Save the screw.

Note: PC users should avoid installing the PCI card in the shared slot (i.e., the PCI slot next to the first ISA slot.

10. Carefully insert the PCI bus interface board into the PCI slot.
11. Secure the metal bracket back into place with the screw from step nine.
12. Put the cover back on the computer.
13. Reboot the computer.
    - **If you have Windows 95**, the computer should initially indicate that it has found a new device. The wording of the messages varies according to which version of Windows 95 you are using.
    - Windows 95 then prompts you to reboot the computer to complete the installation. Click on **Yes** to reboot your computer.
    - **If you have Windows NT**, you will not see any messages of this type because NT automatically updates its registry with the new information.

Connecting the Camera to the Microscope

After you have installed the PCI board, you can connect the SPOT camera to your microscope. All models of the **SPOT Insight** camera require a C Mount coupler. C-mount versions of Diagnostic Instruments' **D10 (1x)** series couplers work with most microscopes. If your microscope will not work with a D10 series coupler, the following options are available:

- **HRU060-CMT coupler (0.60x)** and the appropriate bottom clamp.
- **HRD100-CMT coupler (1x)** and the appropriate bottom clamp.

To attach the camera to the microscope, follow these steps:

1. Attach the bottom clamp to the coupler.
2. Screw the Insight camera into the coupler/bottom clamp until it is tight.

3. Insert the camera/coupler/bottom clamp combination into the microscope's photoport or phototube.
4. Tighten the thumbscrew on the microscope's phototube (or the coupler's bottom clamp) to lock the combination to the microscope.
5. Connect the camera to your computer as follows:

WARNING

MAKE SURE THAT BOTH THE COMPUTER AND THE SPOT POWER SUPPLY ARE TURNED OFF BEFORE CONNECTING THE CABLES. FAILURE TO DO SO CAN RESULT IN DAMAGE TO ELECTRONIC COMPONENTS IN THE CAMERA.

a) Attach one end of the of the 50 pin (10 ft.) cable to the PCI board on the back of the computer, and attach the other end to the camera head.

WARNING

THE 50 PIN CABLE USES THE SAME CONNECTOR AS A PC SCSI PORT. PLUGGING THE CABLE INTO A SCSI PORT WILL DAMAGE BOTH YOUR CAMERA AND SCSI CARD.

b) Attach the power supply/cable to the camera head.

c) Plug one end of the power cord into the power supply and the other end into a surge protector.

Multiple Camera Installations

More than one SPOT camera can be operated from a single computer. The process for installing multiple cameras is simple and applies to both Windows and Mac users. Any combination of SPOT, RT, or Insight cameras can be used.

To install multiple cameras on one machine, follow these steps:

1. Install the software, as specified in **Installing the Software** (see above).
2. Shut down the computer and install ALL of the PCI cards for the cameras that you want to connect to the computer, as described in **Installing the Hardware** (see above).
3. Re-start the computer. When you open the SPOT program, you are prompted to select the camera that you want to use.

Using SPOT's On-line Documentation

To use the on-line documentation that accompanies your SPOT camera, you must first install the Acrobat Reader software that is included on the CD. This software enables you to read and/or print the Portable Document Format (PDF) version of the user guide that is on your CD.

Quick Start

Taking Your First Picture

The following sections list the basic steps for setting up your microscope and camera to immediately begin capturing images in the **Basic** program mode.

The picture taking process consists of two multi-part steps:i

1. Preparing the microscope, specimen, and camera.
2. Capturing the image.

These steps assume that you have already installed the SPOT software and connected the camera to your computer. If you have not done so, refer back to the previous part of this manual, **Installation Guidelines**.

Preparing the Microscope and Camera

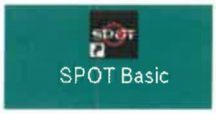
1. Select the specimen that you want to photograph, and set up the microscope for Koehler illumination.
2. Ensure that the specimen is in focus.
3. Using a daylight filter (5500° K), set the lamp voltage to the photo setting (or the rated lamp voltage).

Keep an assortment of ND (neutral density) filters on hand to use for images that are too bright (i.e., you receive the **Exposure time too short for Camera** error message).

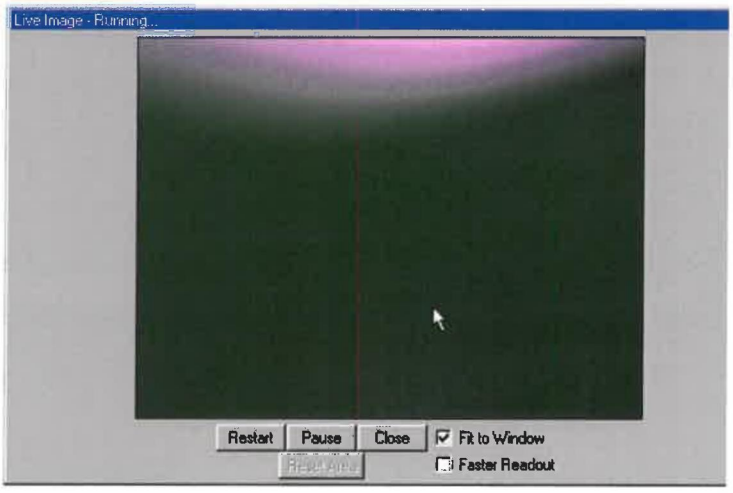
Capturing the Image

To capture an image, follow these steps:

1. **Open the SPOT Basic Program Mode.**
   From the desktop click on the SPOT Basic icon.



2. **View a real-time preview of your image capture.**
   Click on the Live toolbar button. The Live Image window appears. As the camera computes the optimal auto-exposure sequence, the title bar displays **Computing Exposure.** When the program finishes the exposure computation sequence, the title bar displays **Running**, as shown at right



3. **Use the microscope's controls to focus and frame the image as needed.**

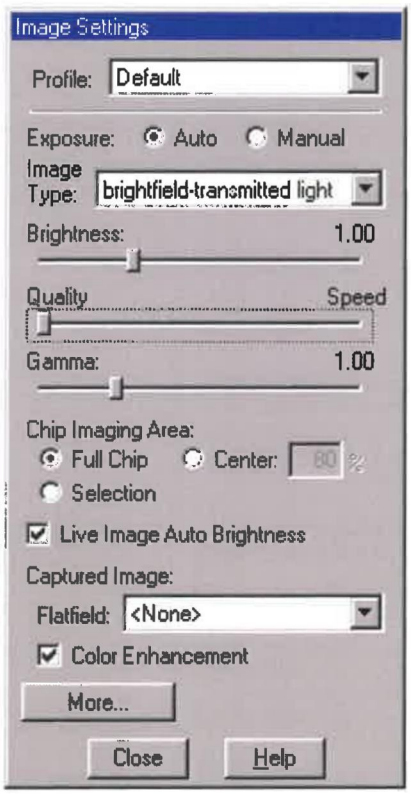
4. **Perform a White Balance on the image.**

   a) Click on the White Balance toolbar button. The live image display pauses, and the Compute White Balance dialog prompts you to **Move to Clear Area of Slide**.

   b) Move the slide so that the light is going through a clear part of the slide.

   c) Click on **Begin**. The Compute White Balance dialog displays **Computing Exposure**, and the Live Image window restarts and computes the new exposure settings.

5. **Click on the Image Settings toolbar button.**
   The Image Settings window appears. Ensure that the settings are as follows:



Main Window:

- **Profile** – Default
- **Exposure** - Auto
- **Image Type** – (your type of microscopy)
- **Brightness** – 1.00
- **Gamma** – 1.00
- **Quality/Speed** – Quality (left-most position)
- **Chip Imaging Area** – Full Chip
- **Live Image Auto Brightness** - Selected
- **Flatfield** – unselected
- **Color Enhancement** – selected

More Image Settings:

- **Image Flip** – as needed
- **Rotate 90°** – as needed
- **Minimum Exposure** – unselected
- **Live Image Maximum** – default (10 sec)
- **Noise Filter** – selected

The suggested settings are designed to give you quality image captures in most, but not all cases. If your work involves fluorescence techniques, for example, you will need to adjust the Image Type, brightness, gamma and Quality/Speed settings accordingly. For more details on selecting Image Settings, refer to Ch. 3 – The Basic Program Mode.

6. **Click on the Get Image toolbar button.**
   The Get Image dialog box displays the status of the capture as it is downloaded to your screen.

Because Get Image uses the values from the live image view, the image capture skips the initial exposure computation sequence.

Ch. 1 - New Features

Version 3.5 of the SPOT software includes the following new features:

Feature	Description
Annotation	• Annotation objects can now be positioned and sized to the exact values that you specify. • The **User Name** Text object has been added to Annotation mode.
Auto-Save	You can now set image capture preferences (Setup>Auto-Save Images) to automatically save during the image capture process.
AVI "movie" file export	SPOT image sequence files can now be exported in AVI file format.
Basic Mode	The Basic mode has been modified as follows: • The Basic mode interface has been redesigned. • Sequential images can now be captured in Basic mode. • A Basic mode TWAIN interface is now available.
Edit	• The **Match Color** dialog has been redesigned. • The **Merge Image** option has been enhanced to include brightness scaling and custom positioning of the merged images. In addition, the Merge Image dialogue now includes an **Apply** button that allows you to view changes without saving the image. In this way, you can continually modify the merged image until it appears as you want it to.
Full Screen mode	You can now exit Full Screen mode by moving your cursor to the top of the screen and clicking on the **Exit Full Screen** button.
JPEG2000	The SPOT software now supports the JPEG2000 image standard.
Palettes	The new SPOT color palettes allow you to apply specific hue and saturation values to monochrome (8 bit or 12 bit) images. Palettes are defined via the **Setup** menu option (see Ch. 4). Palettes can be applied either as part of the image capture process (see Ch.5) - i.e., with the **Palette** option on the Image Setup window – or as a post-capture editing option, i.e., via the **Set Palette** Edit menu option (see Ch. 7).
Reporting	• Report objects can now be positioned and sized to the exact values that you specify. • Static images can now be added to report templates via the **File>Add Image to Report Template** option. Refer to Ch. 4 – Working in Advanced Mode for details. • The **User Name** Text object has been added to Report mode.
TWAIN Interfaces	Preferences are now accessible via both the Basic and Advanced modes.
Zoom	Zoom functionality has changed. The new functionality allows you to add measurements, calibrations, and annotations to zoomed images. Refer to Ch. 4 – Working in Advanced Mode for details.
Annotation	• Annotation objects can now be positioned and sized to the exact values that you specify. • The **User Name** Text object has been added to Annotation mode.

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Ch. 2 – Basic and Advanced Program Modes

Introduction

With the SPOT software you can work in either a Basic or an Advanced program mode.

- The **Basic** program mode is a simplified version of the SPOT program that enables new users to quickly start capturing images without having to go through all of the decision making processes associated with the Advanced version of the program. The Basic program mode is also ideal for users who simply need "snapshots" of their specimens. Refer to Ch. 3 – The Basic Program Mode for more details.

- The **Advanced** program mode is an improved version of the previous SPOT software. It is designed for more advanced users who want both greater control over the way images are captured and edited, as well as access to non-image capture functions such as database image archiving and reporting. Refer to Chapters 4 – 11 for more information on working in the Advanced program mode.

When you install the SPOT software, the installation process adds the Basic and Advanced program modes to your menu structure as well as two icons to the desktop. The following section briefly compares the two program modes.

Basic vs. Advanced Features

The Basic SPOT program includes only basic image capture and editing functionality. The following table compares the general features of the Basic and Advanced SPOT programs.

Features	Advanced	Basic
automated post-processing adjustments	X	X
calibration and measurement functions	X	X
customizable (user-defined) image setups	Extensive	Simple
customizable printing	X	X
database (Windows only)	X	
exposure adjustment	X	X
image annotation	X	X
image editing	Extensive	Simple
live image preview	X	X
image editing in multiple color spaces	X	X
pre-defined image setups	X	X
recall of exposure values used in previous images	X	
reporting	Extensive	Simple
sequential imaging	X	X

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Ch. 3 – The Basic Program Mode

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QUICK REFERENCE:

- Display thumbnails of selected images...p. 30
- Define image capture settings...p. 35
- View a live (real-time) image... p. 43
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- Capture an image... p. 45
- Edit the brightness and/or contrast of a captured image... p. 48
- Add textual and/or graphic annotations to a captured image... p. 52
- Create a simple report... p. 54

Introduction: Using the Basic Program

The Basic SPOT program allows you to quickly and conveniently capture and edit images, without having to make decisions related to the numerous processing and editing options available in the Advanced program.

This chapter is divided into the following sections:

- Navigating in Basic Program Mode
- Preparing to Take a Picture
- Taking a Picture
- Viewing, Editing, and Annotating Images
- Creating Reports
- Printing Options

Navigating in Basic Program Mode

The following sections are an introduction to the SPOT Basic mode operating environment. They include:

- The Viewing and Editing Window - The primary window for all image capture and editing functions.
- Opening and Saving Files - Standard file save and open features, as well as those that are unique to SPOT.
- Setup - Initial settings to customize your software.

The Viewing and Editing Window

When you open or capture an image, it appears in the main viewing and editing window. All of the image save, view, edit, and annotation functions are accessed from this window, which consists of three areas:

- The Menu Bar
- The Toolbar
- The Status Bar

The Menu Bar

The Menu Bar in the Basic program varies according to whether an image is open.

If you have not yet either captured a new image or opened an existing image, the following menus are available:

File
Camera
Setup
Help

If you have either captured a new image or opened an existing image, the following menus are available:

File
Edit
View
Camera
Setup
Window
Help

- The **File** menu contains file open and save options, which for the most part, are identical to the standard file open and save options. Refer to Opening and Saving Files (below) for details. In addition, the File menu contains the report creation and viewing options. Refer to the Basic Mode Reporting section of this chapter for more details.
- The **Camera** menu contains initialization, image capture, white balance, flatfield correction, and live imaging options.
- The **View** menu contains different options for viewing captured images. Refer to Viewing, Editing, and Annotating Images (below) for details.
- The **Setup** menu contains options used to define operating preferences. Refer to Setup (below) for details.
- The **Edit** menu contains image editing options.
- The **Window** menu contains standard Windows display options. Refer to Viewing, Editing, and Annotating Images (below) for details.
- The **Help** menu contains information about the SPOT software and enables you to access the on-line help system.

The Toolbar

The Toolbar appears underneath the Menu Bar and contains all of the image capture, editing, and annotation functions in the Basic program. The following diagram illustrates the Basic program Toolbar, as it appears for an open image:

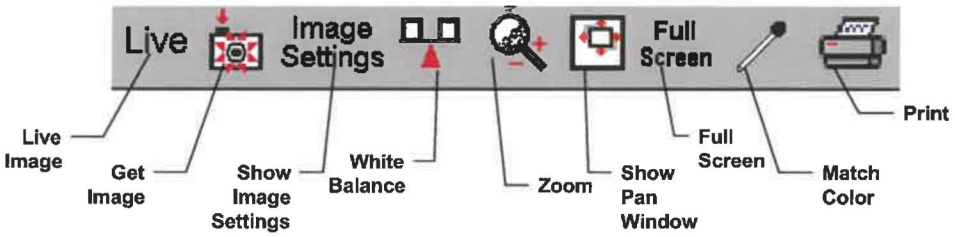


Figure 3- 1: Basic Mode Toolbar

The Status Bar

The status bar at the bottom of your screen displays information about the on- screen image. The following is an illustration of the status bar, as it appears for an open image:

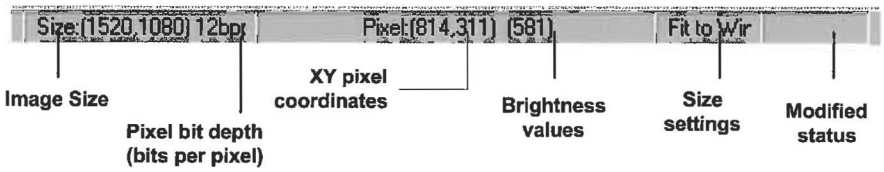


Figure 3- 2: Basic Mode Status Bar

Each of the above call-outs is described in the following list:

- **Image size** – (Columns, rows) in pixels [e.g., (243, 257)].
- **Pixel bit depth** (bits per pixel) - The pixel bit depth at which the image was captured. This value reflects the pixel bit depth for the image setup that was used to capture the image. All images captured in Basic mode are 24 bpp.
- **X Y pixel coordinates** - As you move your cursor over an image, the status bar displays the x y coordinates of the cursor in pixels.
- **Brightness values** - As you move your cursor over an image, the view status bar indicates the brightness values of the pixel coordinates. The status bar displays separate red, green, and blue brightness values for each part of the image [e.g., RGB: (255, 34, 194)].
- **Size Settings** - In the illustration above, the view status bar displays **Fit to Win** (Fit to Window), the default image capture setting.
  - If the Fit to Window option has been deselected, this area displays the word **Normal**.
  - If you are in zoom mode, this area displays the magnification level (e.g., **Mag:x1**, **Mag:x1/4** etc…).
- **Modified Status** - The section to the right of the size settings remains blank until you modify an image. Once an image has been modified from the original status, the word **Modified** appears here.

Opening and Saving Files

All of the file open and save options in the Basic SPOT program are accessed from the File menu. These options are described below.

Note: Standard Windows functions are not described in detail in this manual. For more detail on these functions, refer to the Windows on-line help.

Open Image File

Open image files of a specified format (i.e., JPEG, TIFF, BMP, etc.). In addition to using this menu option, you can open images by dragging an image file from the Windows 95/98/NT/2000 Explorer onto the SPOT program window.

Show Image File Thumbnails

Display thumbnail previews of specified files and open images from the preview screen. To show file thumbnails, follow these steps:

1. From the File menu, select the Show Image File Thumbnails option. The Thumbnail File Spec window displays all of the image files in the directory.
2. Navigate to the directory for the image files that you want to display.
3. Use the **Files of type** drop down box, to specify the file type.

Show Image File Thumbnails (cont.)

4. Select the file or files for which you want to display thumbnails. If you do not select a file or files, the program displays thumbnails for all files of the specified type in the directory.
5. Click on Open. The Thumbnails preview window appears. For each thumbnail, you can:
   - ***Double-click*** (Mac and Windows) on a thumbnail to open an image for viewing or modification.
   - ***Right-click* (Windows)/[[⌘] Click (Mac)** on a thumbnail to display a list of options. The right-click menu enables you to perform a variety of actions directly from the thumbnail window:
     - **Select Image.**
     - **Open Image.**
     - **Delete Image.**
     - **Print Image.**
     - **Move/Rename Image File**
     - **Copy Image**
     - **Add Image to Image Sequence** (see below)
     - **Add Image to Report** (see below)
     - **Select All Images** in the thumbnail view. When you use this option, the right-click menu expands to include the above options for multiple images

   Click and drag with the mouse to select two or more images.

Open Image Sequence File

Open an image sequence file. An image sequence file is a single TIFF format file that contains multiple images.

- Refer to **Taking a Picture** section of this chapter for information on capturing an image sequence file.
- Refer to the Viewing, Editing, and Annotating Images for information on viewing and editing image sequences.

New Image Sequence

Create a new image sequence file. This option enables you to specify the first image in a new image sequence. This image can come from an open image or an image on disk (from file). Subsequent images can be added to the sequence in one of two ways:

- Via the **Add Image to Image Sequence** File menu option (see below). This option allows you either to add an open, non-sequence image to a sequence or to create a new image sequence from the image.
- Via the **Sequence** menu options. Here you can add any image to any point in a sequence. Refer to **Working With Image Sequences** in Ch. 8 for more details.

New Report

Create a new Basic Mode report. Refer to the **Creating Reports** section of this chapter for more details.

Open Report

Open a Basic Mode report. Refer to the **Creating Reports** section of this chapter for more details.

Reload

Reload the image from the hard drive (or other media). All changes made since opening the image file are lost. This option appears only when a saved image is open.

Save

Save a previously unsaved image to new file, or update the changes to an existing file. This option appears only when an image is open.

Save As

Save an image to a new file and/or file location. This option appears only when an image is open.

Save All

Save all open image files. The program prompts you for the name and file location of each image.

Close

Close an image. The program prompts you to specify a location to save the file. This option appears only when an image is open.

Move/ Rename Move and/or rename an image file. This option differs from the Save As option in that it *does not* retain a copy of the original file in the original location: using Move/Rename is comparable to cutting and pasting a file.

Delete Delete an open image. The program prompts you to verify the deletion.

Export Movie File (Windows) Export a SPOT image sequence file to AVI format. Follow these steps:

1. Open a SPOT image sequence. Refer to Ch. 7 – Taking a Picture, for details on capturing image sequences.
2. Select **File>Export Movie File**. The **Export Movie File** appears.
3. Enter the **File Name** for the avi file.
4. Specify the location where you want to save the avi file.
5. Specify which images in the sequence that you want to **Export**. Select either:
   - **All Images** – the avi file will contain all of the images in the sequence.
   - **Specified Images** - the avi file will contain only the images that you specify (i.e., below the radio button).
6. Specify the **Frame Rate** in frames per second.
7. Specify the **Compression Settings**. When you click on the **Compression Settings** button, the **Video Compression** dialog displays the compression application(s) available on your computer. Because each computer will have different installed applications, the Video Compression options will vary.
8. After you have entered all of the desired settings, click on **Save**. The program displays a status message as it exports the file.

Add Image to Image Sequence Add an open, non-sequence image to an open image sequence, or create a new image sequence from an open image. To use this option, the non-sequence image must be the active window and the image sequence must be open in the background.

When you click on **Add Image to Image Sequence**, SPOT prompts you to specify either the **Image Sequence** or a new image sequence. If more than one image sequence is open, select the sequence from the drop-down list.

Add Image to Report Add a selected open image to an open SPOT report. When you select this option, SPOT prompts you to select from a list of reports. If you have not defined a report, SPOT automatically creates a new report for you and inserts the image as a **Static Image** object. Refer to the **Creating Reports** section of this chapter for more details.

Print Print an open image. Refer to Chapter 11 – Printing Images for information on standard Windows and SPOT custom printing features.

Printer Setup Define the page setup parameters. Refer to Ch. 11 – Printing Images for information on SPOT's custom printing features.

Exit (Windows) Quit (Mac) Exit the SPOT program.

Setup

The Basic program mode Setup menu options are as follows:

- Show Image Settings
- Calibration Setup
- Image Auto-Save
- Preferences

Show Image Settings

Use this option to display the **Image Settings** window. In the SPOT Basic Program Mode, the **Image Settings** window is used to define the settings for both the live image view and the final image capture. This means that you can use the Image Settings window in one of two ways:

- As a stand-alone dialog to pre-define the settings that the SPOT camera uses for capture images.
- In conjunction with the Live Image window. When using the Image Settings dialog to define the live image view, the live image settings also serve as the image capture settings. In other words, the live image view can be used as a guide to how the final image will appear.

Refer to the **Preparing to Take a Picture** section below for a description of the options on the Image Settings window.

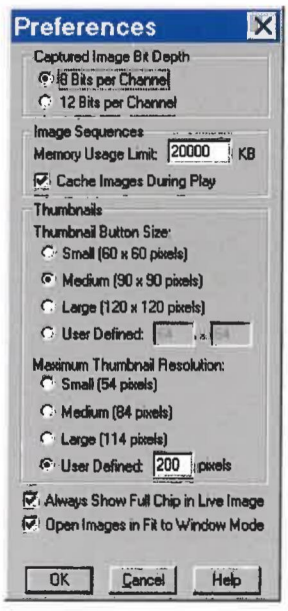
Calibration Setups

With the SPOT program, you can use a stage micrometer to calibrate each objective on your microscope. Once calibrated, you can use the SPOT program to perform basic measurements on an image.

- *For details on calibrating objectives...*
  refer to the **Calibration Setups** section of Ch. 4.
- *For details on adding measurements and/or calibration marks to your images...*
  refer to the **Annotation – Calibration and Measurement Marks** section of Ch. 8.

Image Auto-Save

Use the **Image Auto-Save** feature to automatically save images as part of the capture process. To enable SPOT's auto-save feature, select **Setup>Image Auto-Save**, and check the **Auto-Save Images** box, as shown at right. For details on auto-saving images, refer to the **Image Auto-Save** section of Ch. 4.



Preferences

Use the Preferences option to specify:

- Captured Image Bit Depth.
- Sequential imaging memory settings
- Specify thumbnail appearance.
- Live image viewing settings
- Fit to window view settings
- File type extension setting (*Macs only*).

To open the Preferences window, select **Preferences** from the Setup menu. Each preference is discussed below.

Captured Image Bit Depth

Specify the pixel bit depth for captured images.
The Captured Image Bit Depth option enables you to specify a different image bit depth when saving an image captured in the SPOT program

- **8 Bits per Channel** – The 8 bits per channel option provides 256 gray levels per color:
  - With Insight B/W cameras, captured images are 8 bits per pixel monochrome.
  - With Insight Color cameras, captured images are 24 bits per pixel RGB color.

  This option results in smaller, more manageable image sizes, and is best for images that do not require extensive modification.

- **12 Bits per Channel** – The 12 Bits per channel option provides 4,096 gray levels per color.
  - With Insight B/W cameras, captured images are 12 bits per pixel monochrome.
  - With Insight Color cameras, captured images are 36 bits per pixel RGB color.

  This option reduces banding problems that can result with modified images, but also results in larger file sizes.

  When saving 12 bit per channel images, SPOT prompts you to select either 12 bits per channel or 16 bits per channel. If you use Adobe's Photoshop for image editing, you should select the 16 bit per channel option because Photoshop cannot read 12 bit per channel images.

  Note: The SPOT Insight camera captures all images using 10 bits per channel (color). The SPOT software immediately converts the captured image to either 8 bits per channel (color) or 12 bits per channel (color), depending on the **Captured Image Bit Depth** *that you select.*

Image Sequences

Memory Usage Limit
Specify how much memory is allocated for image acquisition. Note that the entry parameters are system dependent.

Cache Images During Play

When "playing" an image sequence (see Ch. 8), the SPOT program allows you to select how it loads the displayed images:

- *If* ***Cache Images During Play*** *is selected…* The program pre-loads as many images from the sequence as your computer's RAM allows. This enables a faster display of the image sequence. But, if you have a sequence with many images, the size of the combined images could exceed the RAM, and in this case, the SPOT program will load as much of the sequence as possible, play that part of the sequence, and then pause to load the remaining images.

- *If* ***Cache Images During Play*** *is not selected…* The program loads each image in the sequence individually. In cases where you have a large sequence with many files, this allows you to work without being restricted by RAM limitations. The display of images will be somewhat slower than a display where images are cached

Thumbnail Button Size

Specify the size of thumbnails for the Show Image File Thumbnails option

Select from one of the following options:

- **Small (60 x 60 pixels)**
- **Medium (90 x 90 pixels)**
- **Large (120 x 120 pixels)**
- **User Defined**
  Minimum width and height = 20 pixels
  Maximum width and height = 200 pixels

Maximum Thumbnail Resolution

Specify the resolution of image thumbnails (Show Image File Thumbnails option)

Select from one of the following options:

- **Small (54 pixels)**
- **Medium (84 pixels)**
- **Large (114 pixels)**
- **User Defined**
  Minimum resolution = 20 pixels
  Maximum resolution = 200 pixels

Always Show Full Chip in Live Image

Always Show Full Chip in Live Image ensures that you will always see the Full Chip view when viewing a live image. Keep in mind that the selection that you make here determines how the live image displays when viewing a region of the chip. Refer to the **Chip Imaging Area** section for details.

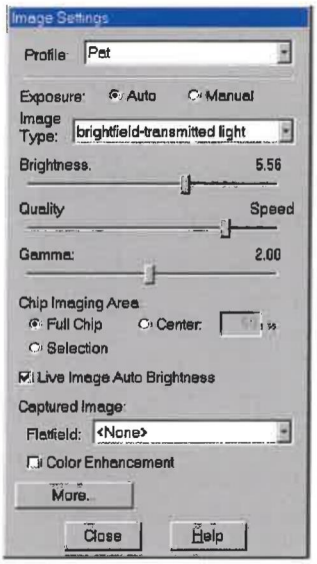
Open Images in Fit to Window Mode

Specify whether images should open in Fit to Window mode.
The Fit to Window option, a program default, automatically sizes images to fit the available screen space when you open or capture an image. Thus, you can immediately see the entire image without having to zoom out. This is especially useful with smaller monitors. Open Images in Fit to Window Mode is checked as a default. If you do not want images to open in the Fit to Window mode, deselect the box.

Add Extensions to File Names on Save (Mac)

When selected, the **Add Extensions to File Names on Save** option automatically appends the file type extension (e.g., JPG, tif, bmp, etc...) to your image file. This enables cross platform file sharing, because Windows based programs require a file type extension, while Mac based programs do not. Once the file type extension is added, Windows based programs can recognize the Mac file.

Preparing to Take a Picture



The **Image Settings** dialog is the central point for defining both live image and image capture settings. The Image Settings window enables you to optimize the live image view, and then capture an image using these settings. To open the Image Settings dialog, either click on the **Image Settings** toolbar button, or select **Show Image Settings** from the Setup menu. Each image capture/live image view option is discussed in the sections below.

Profile

The **Profile** option allows you to create and re-use sets of live image/image capture settings. When you make changes to any of the live image/image capture settings, these changes can be saved to a Profile – either the Default or one that you have defined. For example, if you gamma adjust a live image and then decide that the result is ideal for the type of specimen you are working with, you could create a new Profile with the gamma and exposure values and name it for that type of specimen – i.e., "Diseased Cells."

When you close the **SPOT Camera** window, the program prompts you to save changes. Carefully consider the effect of your choice:

- Clicking **Yes** modifies the Profile according to the adjustments that you made. Future image captures taken with that Profile will reflect these changes. For example, if you rotated a live image, and then clicked Yes after closing the window, all future captures taken with that Profile would be rotated in the same way.
- Clicking **No** retains the original values of the Profile. This allows you to maintain a unique set of image view and capture settings that can be re-applied to future captures of similar specimens.

To add, edit, or delete Profiles, **right-click** on the Profile box. Right clicking gives the following options:

- **Add** – Add a new Profile. When you add a new Profile, the program prompts you to name it.
- **Save** – Save a Profile that you have modified.
- **Rename** – Rename an existing Profile.
- **Delete** – Delete a Profile. This option appears only if there is more than one Profile.

Exposure: Auto or Manual

The **Exposure** option gives you the choice of having the camera calculate exposure time (**Auto**), or setting the exposure on your own (**Manual**). The exposure options will vary according to whether you select **Auto** or **Manual**, as illustrated below. When you enter one of the above exposure settings, note that it applies **both** to the live image that you are viewing and the captured image.

Auto Exposure options

Image Type

An Image Type tells the SPOT program how to expose and process image captures. Each of the 11 available Image Types (see below) corresponds to a microscopy technique: select the Image Type that matches the technique you are using. The camera will capture the image accordingly. The table below describes typical situations for each image type.

Image Type	Description
Brightfield-transmitted light	Stained specimens on glass slides using transmitted light microscopes.
Darkfield-transmitted light	Bright specimens on dark backgrounds using transmitted light darkfield technique.
Fluorescence	Fluorescence specimens not fluorescing in a single red, green, or blue primary color
Nomarksi DIC/Hoffman	Specimens being viewed with either Nomarski DIC or Hoffman techniques.
Phase contrast	Specimens being viewed using the phase contrast technique.
Polarized light	Specimens being viewed with polarized light techniques.
Brightfield-reflected light	Polished metallurgical specimens and semi-conductors using a microscope with a vertical illuminator.
Darkfield-reflected light	Bright areas of specimens being viewed with darkfield, reflected light techniques.
Stereo/macro–no glints	Matte specimens without bright glints.
Stereo/macro–shiny glints	Shiny specimens with bright glints.
Stereo/macro-metallic glints	Metallic specimens with intense glints.

Brightness

Enter a **Brightness** value between **.24** and **34**.
A brightness value of 1, the default value, gives you the correct image brightness for most images. The Brightness control allows you to overexpose or underexpose (darken or lighten) the image area. In many cases, an image has varying levels of brightness; to see a specific area better you might want to lighten or darken the image. When using the Brightness control, the SPOT camera uses an algorithm that combines gain and exposure time to provide the fastest possible image update rate (shutter speed) in live mode:

- When overexposing images to make them brighter (i.e., entering values greater than one), the camera first selects the highest possible gain before lengthening the exposure time. Because gain also brightens an image, less exposure time is needed.
- When underexposing images to make them darker (i.e., entering values less then one) the camera uses the lowest possible exposure time before lowering the gain.

Quality/Speed (Auto)

Use the slider to balance speed and quality when previewing dim images.

- Moving the slider toward the **Quality** side increases image quality and decreases frame rates.
- Moving the slider toward the **Speed** side increases frame rates and decreases image quality.

The Quality/Speed measurement corresponds to the Auto-Gain limit setting which allows you to set an upper limit on the gain options that will be available during the Auto-Exposure process.

- When the slider is at the left-most point, the Auto-Gain Limit is 1, which restricts the camera to a gain of 1, giving you higher quality pictures, but lengthier exposure times.
- When the slider is at the right-most point, the Auto-Gain Limit is 32, which allows the camera to select from the all six gain options. This shortens exposure times, but decreases image quality.

Refer to the "Auto-Gain Limit" section in Ch. 5 – Preparing to Take a Picture for a detailed explanation of gain and exposure.

Gamma

Enter a **Gamma** value between **.1** and **4**.
Gamma adjustment smoothly lightens darker areas of an image without burning out bright areas or lightening black areas. This is especially useful for capturing/editing images with a wide range of brightness values (dynamic range) such as fluorescence specimens or macroscopic objects with glints, (e.g., printed circuitry).

- Values between **1** and **4** lighten the darker areas of the image.
- Values between **.1** and **1** darken the mid-tones of the image.

Gamma adjustments on live images are made in the RGB color space, the only color space where adjustments can be made quickly enough to keep up with live imaging. Refer to Ch. 5 – Preparing to Take a Picture for more details on gamma adjustment and color spaces.

Manual Exposure Options

Exposure Time

Use the slider-bar to specify an exposure time between **.04 ms** and **526.746 sec**.
When you specify an exposure time, the camera skips the automated exposure sequence that takes place when you open the live image view. This is very useful in cases where exposure times are lengthy, as with fluorescence samples.

Gain

Enter the precise gain that the camera will use for an image capture. Use the slider-bar to select one of the six gain options: **1**, **2**, **4**, **8**, **16**, or **32**. The gain value displays to the right of the slider-bar. Refer to the "Auto-Gain Limit " and "Gain" sections of Ch. 5 – Preparing to Take a Picture for details on gain and exposure.

Gamma

The manual Gamma adjustment is identical to the auto Gamma adjustment (see above.)

Chip Imaging Area

SPOT allows you to use any rectangular portion of the CCD chip for image capture. Using a portion of the chip is helpful in cases where you want to:

- Keep file sizes small.
- Shorten download times.
- Avoid areas of the image with optical problems (e.g., vignetting).

There are three image area options:

- **Full Chip** – Capture the entire area of the CCD chip.
- **Center** – Capture a rectangular part of the CCD chip's area that has the same height - to - width ratio as the full chip, and is centered on the center of the full chip. The percentage that you enter is the percentage of full chip width that the new area's width will be.

  To capture a centered proportion of an image, follow these steps:
  a) Check the Center option.
  b) Enter the percentage of the image that you want to capture. The minimum percentage is 10 and the maximum is 100.
  c) Capture the image.
- **Selection** –Capture a specific part of the CCD chip.
  a) Check the **Selection** option.
  b) Close the Image Settings window and open the Live Image dialog (click on the **Live** toolbar button).
  c) Use the mouse to select a region for capture. Click and drag to define the selection.
  d) Capture the image.

When you are viewing a live image **Selection**, how this selection is displayed, depends on whether the **Open Images in Fit to Window Mode** and **Always Show Full Chip in Live Image** preferences (see the **Preferences** section above) are checked. The following table illustrates the possibilities:

Fit to Window Checked?	Always Show Full Chip in Live Image Checked?	Selection Display	Live or Captured Image
YES	**YES**	When you select a portion of the chip, the selection is outlined with a **yellow rectangle**.	**LIVE**
YES	**NO**	When you select a portion of the chip the cursor is a **zoom tool**. To zoom in on a portion of a live or captured image, click and drag over the area that you want to zoom in on. To return to the non-zoomed image, click on the **Full Chip** option.	**BOTH**
NO	**YES**	When you select a portion of the chip, the selection is outlined with a yellow rectangle. However, because **Fit to Window** is not checked, you will not see the entire chip in the window.	**LIVE**
NO	**NO**	When you select a portion of the image, the cursor is a cropping tool. If you select a portion of an image, the program displays only the selected area and discards the rest of the image. To return to the non-cropped image, click on the **Full Chip** option.	**BOTH**

Live Image Auto-Brightness

Available only when you are using **Auto** exposure and viewing a live image, the **Live Image Auto-Brightness** option enables you to maintain a consistent brightness level for changing specimens.

Flatfield

For all other SPOT camera lines, the Flatfield option is used to correct for display problems associated with uneven intensity or coloration in illumination, or to correct for artifacts (e.g., dust) in the optical system. Flatfield can still be used for this purpose with SPOT Insight cameras. But, because SPOT Insight cameras use a color mosaic chip, Flatfield correction serves a new primary purpose. In the production process for color mosaic CCD chips, the RGB colors are applied directly to the pixels on the chip. This process often results in an uneven color density around the outer 30-40 rows of the CCD chip, causing a "halo" appearance in some image captures, particularly those with low contrast. The Flatfield option can be used to correct this problem.

Note: Flatfield correction with the SPOT camera refers only to the correction of uneven lighting. It should not be confused with the correction of optical field flatness.

Flatfield correction works by taking a picture of the brightfield background without the specimen in the field of view. This image lets the SPOT software know, on a pixel-by pixel basis, which areas in the field of view are receiving less illumination than the brightest areas. When taking a picture that contains a specimen, the software uses this information to accurately increase the brightness of each pixel in the dimly illuminated areas. The resulting image gives the appearance of an even illumination.

The Flatfield correction process involves two images - the flatfield image and the final image. To use Flatfield Correct, follow these steps:

1. Ensure that the specimen is under the desired objective and in focus.
2. From the Camera menu, select Get Flatfield Image. The Get Flatfield dialog box appears.
3. Remove the slide from the field of view.
4. Click on Begin. When the program finishes computing the exposure times and acquiring the flatfield file, the program prompts you to name and save the file.
5. Name the Flatfield file. Because flatfield image files are different for each objective, you might want to name the file according to the objective's magnification (e.g., 10x).
6. Click on OK. If you do not specify a directory path, the program automatically saves the flatfield file:
   - In Windows, the file is saved to the **c:\SpotCam\Fltflds** directory
   - In the Mac OS, the file is saved to **System Folder>Preferences>SPOT 3.2 Prefs>Flatfields**.

Note: The default file location varies, depending on where you installed the SPOT program.

7. Move the specimen back into the field of view.
8. Click on the **Live** toolbar button.
9. Open the **Image Settings** window, and select the Flatfield box. The name of the flatfield file that you defined in step four should appear in the drop-down list of flatfield files.
10. Perform the image capture. SPOT automatically uses the flatfield image to correct the capture.

Refer to the Taking a Picture section of this chapter for more details.

Color Enhancement (Captured Images)

The Color Enhancement option optimizes image captures by closely matching the final capture to the image as seen through the microscope. To optimize the final image, the SPOT program processes the raw image data to produce a final image that matches the view seen through the microscope.

If you want an image capture to match the live image view, you will want to leave Color Enhancement off. Color Enhancement applies only to captured images.

More (Additional Orientation and Exposure Settings)

When you click on **More**, the program displays a dialog of additional orientation and exposure settings, as shown at right.

Flip

Specify whether images will be flipped during the image capture/live image viewing process.

- Select **Horizontal** to flip the image from left to right.
- Select **Vertical** to flip the image from top to bottom.
- Select both options to flip the image from left to right and from top to bottom.

Rotate 90°

Specify whether images will be rotated during the image capture/live image viewing process.

- Select **None** if you do not want the image to be rotated.
- Select **Right** to rotate the image clockwise 90 degrees.
- Select **Left** to rotate the image counterclockwise 90 degrees.

Exposure Limits - Minimum

Because SPOT Insight cameras are capable of extremely short exposure times, light sources with intensities that vary with the waveform of the 60 Hz line supply (e.g., fluorescent lamps, 120 volt incandescent bulbs, and 6 volt 20 watt halogen lamps) can cause the Live Image window to flicker and result in incorrect brightness and color balance readings in captured images. The **Minimum Exposure** option allows you to correct for these problems by limiting the camera to a minimum exposure time that is greater than the pulse interval of the light source. Consider the illustration to the right. In this case, the pulse interval of the fluorescent light source is 8.3 ms. Entering a

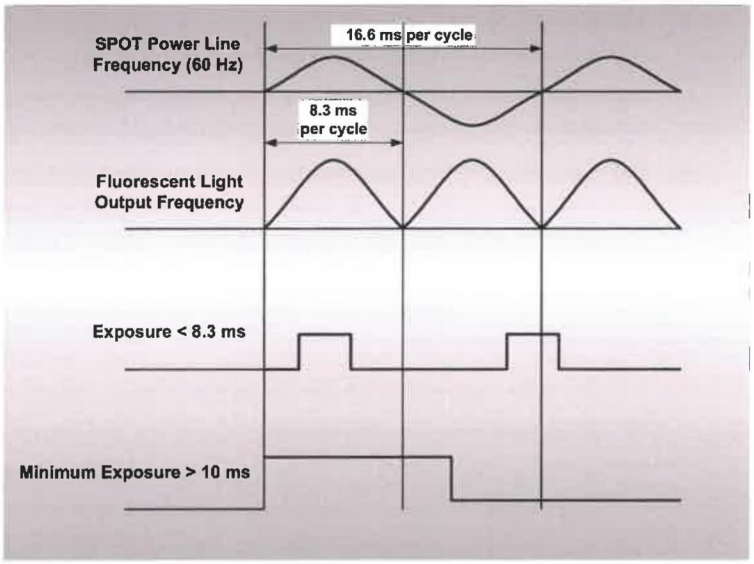


Figure 3- 3: Setting a Minimum Exposure to Accommodate Varied Light Sources (Basic Mode)

Minimum Exposure of 10 ms ensures that the camera's exposure time is greater than the pulse interval of the light source. To enter a minimum exposure, check the Minimum Exposure box, and enter a value in milliseconds.

Exposure Limits – Live Image Maximum

The Live Image Maximum option helps you to avoid unnecessarily long exposures when viewing a live image. Consider the following situation: When you change from a lower resolution objective to a higher resolution objective, the camera detects a brief interval of blackness. When the Auto-Brightness option (see above) is enabled, it compensates by modifying the exposure time to maintain a constant brightness level. This results in you having to wait for the camera to compute a lengthy and incorrect exposure for the black interval. Using the **Live Image Maximum** option, you can limit this exposure time. For most cases, Diagnostic Instruments recommends a value of 0.5 seconds. Similarly, the Live Image Maximum option can also be used in cases where the microscope's light path selector is incorrectly set.

Noise Filter

The Noise Filter corrects for the effects of electrical or thermal noise. When capturing dim images with lengthy exposure times, the noise filter acts as a "virtual cooler" for the Insight camera, eliminating "hot pixels."

The Noise Filter checks each pixel on the CCD chip as described below:

1. The program compares the value of each red pixel with the values of the eight neighboring red pixels.
2. If none of the eight neighboring red pixels has a value within ±15 % (i.e., the threshold value that the SPOT program uses) of the value for the red pixel being checked, the value for the red pixel being checked is replaced with the average value of the eight neighboring red pixels.
3. The program repeats this procedure for the values of the green and blue pixels being checked.

To activate the Noise Filter, check the Noise Filter box. The program runs the noise filter each time an image is captured.

Taking a Picture

The actual capture of a digital image (taking the picture) is similar to traditional photographic techniques. In digital photography, however, it is possible to continually capture, refine, and recapture images until you have an optimal representation: the first image that you capture might be only one of several that you use to produce a publishable product. A sample image capture process, from start to finish might proceed as follows:

1. Adjust the Preferences as needed (see above).
2. Open the Live Image window.
3. Frame and focus the image as needed.
4. Click on the Image Settings toolbar button to access the Image Settings window and adjust the live image view settings.
5. Click on the Get Image toolbar button to capture the final image.

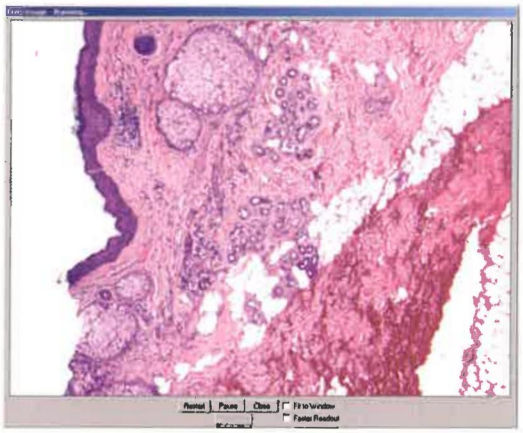
In the Basic program mode, image captures and live image view settings are defined from the same source - the **Image Settings** window. This gives you the convenience of being able to view a live image, adjust the settings in real-time, and then capture the image with the live image view settings.

Note: Except for the **Captured Image Bit Depth**, *which is defined on the* **Preferences** *window, all image capture/live image settings are defined from the Image Settings window.*

Initialize Camera

If you turned on the SPOT power supply after you opened the program, you need to initialize the camera in order to activate all of the capture and editing options. To do so, select the Initialize option from the Camera menu.

Viewing a Live Image



The Live Image option enables you to see a real time preview of your specimen/image capture. For instance, you can use the Live Image window to:

- Scan the image for an area of interest.
- Zoom in on an area of interest.
- Focus on an area of interest.
- Frame an area of interest.
- Define image capture settings – see **Preparing to Take a Picture** above for more details

Note: With some video cards, you might experience system crashes or display problems when working with live images. To correct these problems, lower the Graphics Acceleration setting in Windows, as described in the Video Card Related Problems *section of the* Installation Guidelines *chapter.*

To open the Live Image window, either click on the Live Image toolbar button, or select the Live Image option from the Camera menu.

Note: Occasionally, when viewing a dim live image, you might notice that the right half of the image is brighter or dimmer than the left half. To display images in real time, the camera uses two separate amplifiers to simultaneously read the right and left halves of the CCD chip. With some images, particularly dimmer samples, the brightness of the two halves may not match. This results in a "split" appearance. To avoid this problem with captured images, the camera reads the whole CCD through a single amplifier. A "split" Live Image view is **not** *indicative of what your image capture will look like. Refer to* Appendix A: Electronic Imaging Theory and SPOT Cameras *for more details.*

- The title bar at the top of the Live Image window displays the status of the live image view - **Running, Computing Exposure**, or **Paused**. Double-click on the title bar to "roll up" the live image display. This pauses the live image display. Double-click again to display the whole window and resume the live image display.
- When the **Fit to Window** option is checked, the live image window **cursor** becomes a **zoom tool**. To zoom in on a portion of a live image, click and drag over the area that you want to zoom in on. To return to the non-zoomed image, click on the **Reset Area** button on the bottom of the window.
- When the **Fit to Window** option is not checked, the cursor is a **cropping tool**. If you select a portion of an image, the program displays only the selected area and discards the rest of the image. To return to the non-cropped image, click on the **Reset Area** button on the bottom of the window.

Note: Due to a flaw in the Windows operating system, monitors that are set to higher resolutions cannot zoom to very high magnification levels. If zoomed in to a high magnification, the monitor displays white instead of the zoomed image.

- The **Full Screen** option (accessed via the View menu or [F 7]) can be used with live images. This option allows you to use the entire screen to view a live image, without the SPOT menus/toolbar and the Windows navigation bar.
  - To access live image viewing options in the full screen view, right click on the live image.
  - To exit the full screen view, either press [F 7], or move the cursor to the top of the screen and click on the **Exit Full Screen Mode** button that appears.

- There are six **view/exposure options** at the bottom of the Live Image window:

Restart	Resample the brightness of the image, compute new exposure times, and restart the live image display. This is useful for updating exposure times when specimen brightness has changed.
Pause/Resume	Freeze the live image window. This is useful when you are changing a specimen and want to preserve the last view. In addition, to use the Transfer option (see below), you must first Pause the live image view. Click on Resume to restart the live image view
Close	Close the Live Image window.
Fit to Window	Size images to automatically fit the resizable display window.
Reset Area	Return to the non-zoomed view of the live image.
Faster Readout	Faster Readout enables you to "speed up" the live image display (higher frame rates) in cases where you are willing to sacrifice some resolution for a higher frame rate.

Computing White Balance Values (Insight Color)

White balance is the ratio of red, green, and blue gains necessary to achieve the proper color rendition for an image. Many factors impact the coloration of an image capture, including lamp voltage, the coloration of the glass used in the slide, and the coloration of the glass used in the lenses of the microscope's objectives. To minimize the impact of these factors, use the Compute White Balance feature. When you perform a white balance calculation, SPOT samples the color of the light from your light source after it has passed through the slide and the objective, and then calculates the red, green, and blue gains needed to produce images where the white areas are white and the color areas are true.

You should perform a new white balance calculation when:

- Starting a new image capture session.
- Changing the lamp voltage.
- Switching to a different objective on the microscope.

To compute new white balance values, follow these steps:

1. Set the illumination and the objective that you will use for the image capture.
2. If you have not done so, select your **Image Type** (see above). Each Image Type has its own set of white balance values. Any white balance values that you compute for an image type remain linked to that image type until you compute new values. When selecting a new image type, remember that you will be using the white balance values linked to that image type.
3. Click on the **White Balance** button.

4. Show the camera a sample of white light. This step varies according to microscopy technique and Image Type (sample), as shown in the table below:

Image Type	White Balance Technique
Brightfield-transmitted light	Position the slide so that the specimen is not in the field of view, but light is going through the slide near the specimen.
Darkfield-transmitted light	Perform the white balance using the brightfield mode on your microscope. See Brightfield – transmitted light above.
Fluorescence	Use the Match Color feature (see above)
Nomarksi DIC/Hoffman	**For Nomarski DIC:** Adjust the Nomarski prism until the image is in either the "brightfield" or the "gray scale" view. **For Hoffman:** Perform the white balance directly on the Hoffman image.
Phase contrast	Position the slide so that the specimen is not in the field of view, but light is going through the slide near the specimen.
Polarized light	Pull all polarizers and compensators out of the light path and then position the slide so that the specimen is not in the field of view, but light is going through the slide near the specimen.
Brightfield-reflected light	Replace your sample with a mirror.
Darkfield-reflected light	Perform the white balance using the brightfield mode on your microscope. See Brightfield – reflected light above.
Stereo (all three options)	Using an EPI-illuminator (shining a light on the top of your specimen from off to one side), replace your sample with a white sheet of paper.

Click on **Begin** to start the calculation. The camera computes the white balance values, and they are automatically saved to the image settings for the image type that you selected.

Capturing the Final Image

Get Image captures an image according to the Image Settings that you defined (see previous sections). To capture an image, either click on the Get Image button, or press **[F 9]**.

The Get Image status dialog box appears. Status messages display as the camera computes the gain and exposure times. If the live image window is open, the image capture uses the gain and exposure times calculated for the live image and skips the exposure computation (step 1 below). The status messages are as follows:

1. **Computing Exposure** – The camera is sampling brightness levels in the image in order to determine proper gain and exposure time.
2. **Getting Image** – The camera is downloading the image to the computer.
3. **Shutter open 0:07** – A countdown clock displays when the exposure will last longer than 2 seconds. This informs the user that the electronic shutter is open and counts down the minutes:seconds remaining until it closes.
4. **Processing** – The camera is preparing to display the image.

The newly captured image appears on screen.

Get Sequential Images

The Get Sequential Images option allows you to capture a sequence of images. SPOT's sequential image capture process enables you to save an image sequence in two different ways:

- You can save a sequence as a group of individual image files.
- You can save a sequence as a single (TIF format), "sequence image file" that is composed of multiple images. Refer to the Viewing, Editing, and Annotating Images section for details on viewing and modifying image sequence files.

Refer to Ch. 7 – Taking a Picture for details on capturing sequential images.

Viewing, Editing, and Annotating Images

After capturing an image, you can adjust viewing options, and edit/annotate the captured image.

View

All of the image viewing options are accessible from two menus:

- **View** – Contains options specifically designed for viewing microscopic specimens
- **Window** – Contains standard Windows options that enable you to work with and view multiple images.

Note: The Window menu options include Cascade, Arrange Horizontally, Arrange Vertically, Arrange Icons, and Close All. For descriptions of these features, refer to the standard Windows help.

To help you view and analyze your image captures, the View menu contains five options – **Fit to Window**, **Zoom**, **Show Grid Lines**, **Show Pan Window**, **Image Date/Time**, and **Full Screen**.

Note: The Show Grid Lines option is available only when you zoom in on an image.

Fit to Window

Fit to Window allows you to view the whole image, regardless of the window size. Typically, reducing the size of a window also cuts off part of the image. The Fit to Window option solves this problem by resizing the image so that it is always the same size as the window.

Zoom In/Out

1. Either select Zoom In/Out From the View menu, or click on the Zoom toolbar button:
2. The cursor changes to a magnifying glass, and the Zoom toolbar button becomes active. The precise reference point for the zoom location is indicated by the white dot in the center of the magnifying glass. The row and column numbers at the bottom of the screen indicate the exact position of the white dot as you move the magnifying glass over the image.
3. Use the mouse to zoom in or zoom out:
   a) Position the mouse over the center of the area that you want to zoom in on.
   b) Either left click to zoom in 2x, or right click to zoom out 2x.
   c) To return to the normal view mode, de-select the Zoom option.

The Zoom feature works in conjunction with the pan window (see **Show Pan Window** below), which allows you to see where you are in relation to the larger image while zoomed in.

Note: All of the standard save, view, and edit options, with three exceptions – **Add Calibration Mark**, **Add Measurement**, *and* **Annotate** *– can be used while the program is in zoom mode. If you need to annotate a zoomed image, use the* **Zoom Level** *option (see below).*

Note: With some video cards, you might experience display problems when zooming in on captured images. To correct these problems, lower the Graphics Acceleration setting in Windows, as described in the Video Card Related Problems *section of the* Installation Guidelines *chapter.*

Zoom Level

The **Zoom Level** option, like the **Zoom In/Out** option enables you to view zoomed images. However, unlike the Zoom In/Out option, you have the option to add annotations, including text, calibration marks, and measurements, to the zoomed image. To use the Zoom Level option , select **Zoom Level** from the View menu and select a percentage option from the list.

Like the Zoom In/Out option, the The Zoom Level option can be used in conjunction with the pan window (see **Show Pan Window** below), which allows you to see where you are in relation to the larger image while zoomed in.

Note: With some video cards, you might experience display problems when zooming in on captured images. To correct these problems, lower the Graphics Acceleration setting in Windows, as described in the Video Card Related Problems *section of the* Installation Guidelines *chapter.*

Show Grid Lines

Show Grid Lines clearly defines pixel borders, which are often invisible in low contrast areas of an image. Clearly defined borders help in positioning the cursor at an exact row and column location (i.e., using the readouts at the bottom of the screen), which then allows you to view the brightness value for the individual pixel.

To show the grid lines on an image, follow these steps:

1. Position the magnifying glass in the center of the area that you want to zoom in on.
2. Zoom to either 8x or 16x. If you are using the **Zoom Level** option, select a percentage of 800 or greater.
3. From the View menu, select the Show Grid Lines option. Grid lines appear around each pixel.

Show Pan Window

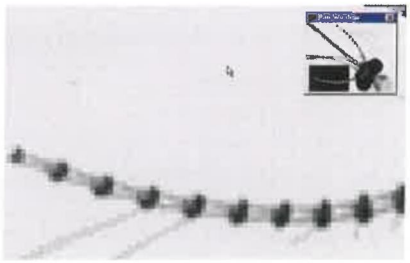
Show Pan Window enables you to navigate around an image while zoomed in at high magnifications. When you select the Show Pan Window option, the program highlights your location in relation to the whole image, and, by dragging the highlighted area around the pan window, you can explore the image, piece by piece. Without the Show Pan Window option, the process of moving to a new point while in high magnification would involve several steps:

1. Zooming in to the first area of interest.
2. Zooming out to determine where you are.
3. Determining a new area of interest.
4. Re-zooming in to the new area of interest.

This tedious process is replaced by the pan window. When you select Show Pan Window, a smaller version of the image appears with a color negative over the zoomed area of the image.

To use the pan window, follow these steps:

1. Either select Show Pan Window from the View menu, or click on the Show Pan Window toolbar button.



Note: The pan window can be opened at any level of magnification.

The pan window appears, superimposed upon the image.

2. Position the cursor on the image in the pan window: it changes from a magnifying glass to a four arrow pointer.
3. To move to a different area of the image, either left click on the rectangle and drag it to the new location, or left click on the new location.

Image Date/Time

The Image Date/Time feature pulls up the date and time associated with the capture of an image. When you capture an image, the SPOT software remembers the date and time of the image capture. When you save the image, even if this is many hours later, the program sets the **Created** date and time of the image file to the date and time of the original image capture.

When image files are transferred over networks, the **Created** date and time is sometimes reset to the date and time of the transfer, making it an unreliable indicator of the image capture time. Because TIFF and JPEG format images have tags both for the Created date and the original capture date and time, they are not affected by network transfers. If reliable image capture date and time information is important to your work, you should save images in either TIFF or JPEG format.

When you select the Image Date/Time option from the View menu, a dialog box displays the date and time associated with the capture.

- For non-TIFF, non-JPEG format image files, this is the **Created** date/time, as explained above.
- For TIFF and JPEG format image files, this is the TIFF/JPEG tag date and time.
- For SPOT Database images (PCs only) this is the original date and time that was stored in the database file.

Full Screen

View a full screen version of an image – i.e., without the SPOT menus or toolbars or the Windows navigation bar. This option allows you to utilize the entire window for previewing images. Press **[F 7]** to view a full screen image or click on the **Full Screen** toolbar button. Press **[F 7]** again to exit the full screen view. Alternatively, Windows users can exit the full screen view by moving the cursor to the top of the window and clicking on the **Exit Full Screen Mode** button that appears.

While working in Full Screen mode, Windows users can access SPOT menu options using standard keystroke combinations, as illustrated in the table below:

[Alt] + A =	Attributes (Report mode)	**[Alt] + H** =	Help
[Alt] + C =	Camera	**[Alt] + O** =	Object (Report mode)
[Alt] + D =	Database (Advanced mode)	**[Alt] + Q** =	Sequence
[Alt] + E =	Edit	**[Alt] + S** =	Setup
[Alt] + F =	File	**[Alt] + V** =	View
		[Alt] + W =	Window

Edit

All of the editing options in the Basic program mode are accessed via the Edit menu. There are five editing categories in the **Basic** program mode:

- Basic editing
- Image position
- Image quality
- Calibration and Measurement
- Annotation
- Memos

The options associated with each category are described below. In some cases, where the options are identical to those in the Advanced program mode, the manual refers you to the appropriate chapter and section.

Basic Editing

The Edit menu contains several functions that are common to most operating systems.

Undo Undo the last edit that you made. The Undo option only allows for one level of edits: in other words, you can only undo the immediately preceding edit. To Undo an action, select Undo from the Edit menu.

Cut (Mac) Cut text fields within the SPOT program.

Copy to Clipboard (PC)/Copy (Mac) Copy all or part of an image onto the clipboard for insertion into another program. These options are identical to the Windows **[Ctrl C]** command and the Mac **[⌘ C]** command.

1. Press and hold the left mouse button (PC)/mouse button (Mac).
2. Drag the cursor over the image until the dotted rectangle covers the area of the image that you want to copy. If you copy without "cutting out" a specific portion of the image, SPOT selects the entire image.
3. Either select the Copy to Clipboard/Copy option from the Edit menu, or press **[Ctrl C]** (PC)/**[⌘ C]** (Mac). The image is now ready to be pasted into another application.
4. Open the application that you want to paste the image into, and select from the available pasting options.

Paste (Mac) Paste text fields in the SPOT program. The Paste field is grayed when editing images.

Clear (Mac) Clear a text field in the SPOT program. The Clear field is grayed when editing images.

Select All (Mac) Select an image for copying into another application.

Image Position

From the Edit menu, you can:

- Re-position images
- Match the on-screen orientation of a specimen to the microscopic view
- Crop an image to better reflect the area of interest

Rotate Right Rotate an image clockwise in 90 degree increments.

Rotate Left Rotate an image counter-clockwise in 90 degree increments.

Flip Horizontal Flip an image from right to left

Flip Vertical Flip an image from top to bottom.

Crop Cut out a portion of an image. To crop an image, follow these steps:

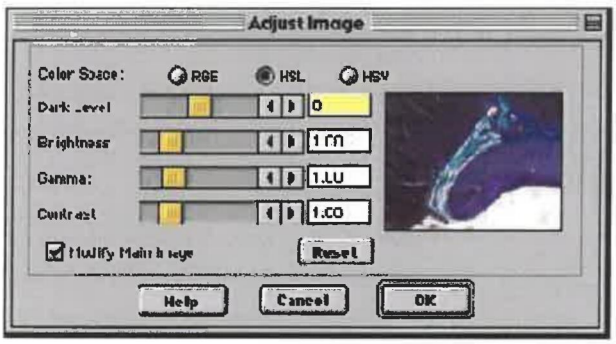
1. Press and hold the left mouse button.
2. Drag the cursor over the image until the dotted rectangle covers the area of the image that you want to crop.
3. From the Edit menu, select Crop. The Crop Area window displays the pixel coordinates of the rectangle that you selected.

From this window you can fine tune the crop area by modifying the pixel coordinates. If you select the Crop option directly from the menu (i.e., without specifying a crop area), the Crop Area window displays the values for the entire image.

4. Click on OK to crop the image. The cropped image appears on-screen.
5. When you crop an unsaved image, the program automatically discards the area of the image outside the crop. To give yourself the opportunity to change the crop area, you should save the image before you crop it.

Image Quality (Adjust Image)

The Adjust Image options allow you to correct images that are overly bright or dark, or to modify areas of interest that are too light or too dark to see important details. To access these editing options, select **Adjust Image** from the Edit menu. The Adjust Image window appears, as shown at right. Adjust images either by entering a value in the box or by moving the slider bar.



The Adjust Image window provides three adjustment parameters:

- **Color Space** – This option enables you to select the color space that is best suited to the type of image that you are editing:
  - **RGB** (Red Green Blue) – Use for monochrome images
  - **HSL** (Hue Saturation Luminance) – Use for all color images except fluorescence
  - **HSV** (Hue Saturation Value) – Use for fluorescence images

  Refer to the **Image Editing and the Concept of Color Space** section of Ch. 8 for more detailed descriptions of the three color spaces.
- **Modify Main Image** - When this box is checked (the default), both the thumbnail image and the main image change as you are adjusting the image. If you want the main image to change after you have made the adjustments – and thereby speed the editing process - deselect the Modify Main Image box.
- **Reset** – Click on the Reset button to return the image to its pre-adjustment appearance.

The following sections describe the effect of each of the four adjustments.

Dark Level The Dark Level adjustment lightens or darkens an entire image by adding the value that you specify to all of the pixels in the image.

To adjust Dark Level, enter a number between -100 and 100.

- Numbers between zero and –100 darken the image.
- Numbers between zero and 100 lighten the image.

Brightness The Brightness adjustment lightens or darkens the bright areas in an image. For example, if you used the Dark Level adjustment to modify an image, but the new bright areas are either too dark or too light to see the details, you can you use the Brightness feature to correct this. Because the Brightness adjustment is *multiplicative* (as opposed to the Dark Level adjustment which is *additive*), it multiplies the pixel values in the image by the value that you specify. A multiplicative process affects bright areas more than dark areas. Before adjustment an image's Brightness value is always one.

To adjust Brightness, enter a number between 0 and 5.

- Numbers between zero and one darken the image.
- Numbers between one and five lighten the image.

Gamma The Gamma adjustment lightens the darker (mid-tone) areas of an image without burning out bright areas or lightening black areas. This is useful when modifying images with a wide *dynamic range*, (i.e., the range of brightness values recorded by the CCD chip. Before adjustment, the Gamma value is always one.

To adjust Gamma, enter a number between .1 and 4.

- Numbers above one lighten the mid-tone areas in the image.
- Numbers below one darken the mid-tone areas in the image.

Contrast The Contrast adjustment exaggerates or subdues the difference between the bright and dark areas in an image. Before adjustment, the Contrast value is always one.

To adjust Contrast, enter a number between 0 and 5.

- Numbers above one exaggerate the contrast between bright and dark areas.
- Numbers below one subdue the contrast between bright and dark areas.

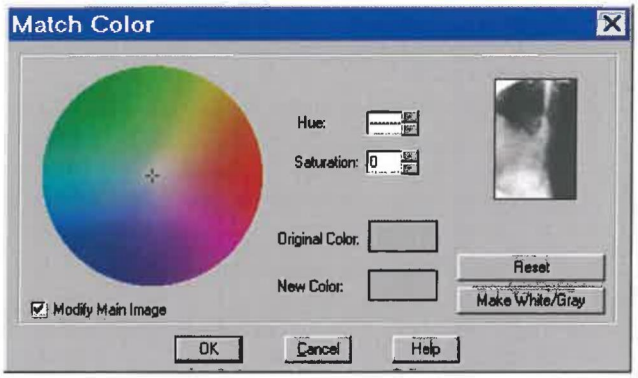
Match Color

The Match Color feature is typically used in two situations:

- You want to change the colors of an image on the screen to match the way that you think the specimen looks.
- You want to set the white balance for pictures taken with a microscopic technique that never has white in its images. A prime example of this situation is fluorescence microscopy.

To use the Match Color feature, follow these steps:

1. From the Edit menu, select **Match Color.** The cursor becomes an eye-dropper.
2. Use the mouse to move the eye-dropper to the pixel location of the color that you want to match. Refer to the status bar at the bottom of the window to ensure that each of the R, G, and B values are less than 255.
3. Click on the pixel. The Match Color window appears.



> *Note: The Set White Balance button appears only for image captures that have not yet been saved and closed.*

The window displays information about the pixel that you selected:

- **Hue** – The actual color, as distinguished from others in the color spectrum. Hue is derived from a color wheel and is expressed in the color's angular location (e.g., 0 to 360 degrees).
- **Saturation** - The proportion of perceived pure hue in the color. Saturation (in the SPOT software) is measured on a numerical scale of 0 to 100, where zero equals pure gray and 100 equals pure hue (e.g., "day glow").
- **Original Color** – A sample "swatch" of the color that you selected. The cross hairs on the color wheel (e.g., in the center of the window) indicate the relative position of the selected color.
- The **Modify Main Image** box is checked as a default.

4. Set the new color. This can be done in one of two ways, depending on whether the image should contain white:
   - If you are trying to make the selected pixel in your image appear as white (or gray if the pixel is darker), click on the **Make White/Gray** button.
   - If you are working with an image that does not have any white, modify the Hue and Saturation values so that the **New Color** "swatch" shows up as the color that you want. You can do this either by entering specific Hue and Saturation values, or dragging the cross-hairs on the color wheel to the desired location.

Calibration and Measurement

The **Add Calibration Mark** and **Add Measurement** options enable you to add calibration and measurement references to SPOT images. Refer to the **Annotation – Calibration and Measurement Marks** section of Ch. 8 for detailed steps.

Annotation

Image annotation is useful when you want to prepare captures for formal presentation, or inclusion in an article or reference work. Using SPOT's image annotation features, you can:

- Add text to an image.
- Use an arrow, line, rectangle, or ellipse to emphasize a particular area in an image.
- Add a graphic stamp such as a company logo to an image.
- Add freehand lines to an image.

To annotate an image, select Annotation from the Edit menu. Several changes take place in the SPOT image editing environment:

- The **Edit** menu options change to reflect annotation editing.
  Refer to **Editing Annotation Objects** *(Ch. 8) for descriptions of each option.*
- The **Object** menu appears with options for inserting drawing objects, text, images, and image related information onto an image. Cursor functionality in SPOT reports varies according to which **Object** menu option is selected.
  - If **None** is selected, the cursor is a an editing tool for selecting, sizing, and positioning objects. When creating new reports, **None** is the default option.
  - If a report object is selected, the cursor changes to a cross-hair icon for placing new report objects on the grid. *Refer to* **Adding Annotation Objects to SPOT Images** *(Ch. 8) for descriptions of each object.*
- The **View** menu changes to reflect annotation mode:
  - The two zoom options and the Show Grid Lines options are no longer displayed..
  - The **Show Tools** option is present – this option displays the Tools dialog box, which contains shortcuts for inserting annotation drawing objects.
    Refer to **Adding Annotation Objects to SPOT Images** *(Ch. 8) for more details on using the Tools dialog box.*
- The **Attributes** menu appears with options for specifying annotation qualities.
  Refer to **Defining Annotation Attributes** *(Ch. 8) for descriptions of each option.*
- The **Done!** menu (Windows)/**Mode** menu (Mac) allows you to switch back to the image capture/edit mode.
  - **Windows** – The Done! menu has only one option, **End Annotation** Mode. When you select this option the SPOTprogram prompts you to merge the changes into the image.
  - **Mac** – The Mode menu has only one option, **Exit Annotation Mode**. When you select this option, the SPOT program prompts you to merge the changes into the image.

CAUTION!

If you select Yes after clicking on Done! The annotations become a permanent part of the image. If you want to preserve the original image, save the original image to a file. After annotating it, use Save As to save the annotated version to a different file.

Duplicate Image

Click on the Duplicate Image option to create a copy of the active (on-screen) image.

Memos

In the SPOT software, you can attach a memo to TIFF or JPEG format image files. To use the Memo option, follow these steps.

1. From the Edit menu, select Memo. The Image Memo window appears for you to enter a new memo or edit an existing memo.
2. Enter the message.
3. Click on OK to attach the memo to the image.

Note: If you attach a memo to an image that is not in either TIFF or JPEG format and then attempt to save the memo (i.e., when you close the image file), the SPOT program displays a warning message that asks you if you want to save the image without the memo. If you want to retain the memo, click on Cancel and use the **Save As** *option to save the image in either TIFF or JPEG format.*

Working with Image Sequences

Image sequence files can be edited in the following ways, as described below:

- The **Sequence** menu – Rearrange the order of a sequence, add and/or remove images to and from the sequence, extract images from the sequence, and play the sequence. Refer to the **Sequence Menu Options** section of Ch.8 – Viewing, Editing, and Annotating Images for details.
- The **Sequence Controls (Image Sequence)** dialog – Set view options and "play" the sequence. Refer to the **Image Sequence Controls** section of Ch.8 – Viewing, Editing, and Annotating Images for details.

Creating a Report

Using the Basic mode's built-in reporting functionality, you can collect, organize, and present information related to your image captures. This chapter discusses the process of creating and printing reports. It is divided into the following sections:

- **Creating a New Report**
- **Working in Report Mode**– general reporting features and functionality
- **Defining View Options**– the view of the report definition screen
- **Defining Report Attributes** – the style and appearance of the report
- **Defining Report Objects** – the text, field, and image information that appears in the report
- **Editing Report Objects** – edit options for the report objects
- **Saving a Report**
- **Printing and Distributing Reports**

Creating a New Report

To create a new SPOT report, select **New Report** from the File menu. The following changes take place in the main window:

- The report definition window appears as a separate window. If an image or image(s) were open, they remain open. When you move between an image window and the report definition window, the menu options change accordingly.
- The **Object** menu appears. Use these options to specify the fields, text, and images that appear in the report.
- The **Attributes** menu appears. Use these options to define the appearance and style of the report.

- The **View** menu options change to reflect report definition function.
- The **Edit** menu options change to reflect report editing functions. These options are available only after one or more report objects has been added to the New Report window.

Working in Report Mode

The report mode contains functionality that differs from the image capture and editing functionality. The following is a brief overview of these features.

Cursor Functionality

Cursor functionality in SPOT reports varies according to which **Object** menu option is selected.

- If **None** is selected, the cursor is a an editing tool for selecting, sizing, and positioning objects. When creating new reports, **None** is the default option.
- If a report object is selected, the cursor changes to a cross-hair icon for placing new report objects to the grid.

Sizing and Positioning Report Objects

When sizing and positioning a report object in the report window, you must first select the object by single clicking on it. When an object has been selected, handles appear at the corners; for line objects, the handles appear at each end. You can select multiple report objects by holding the mouse button down and dragging the cursor around both objects. Handles appear around both objects. When selected, report objects can be sized or positioned as needed.

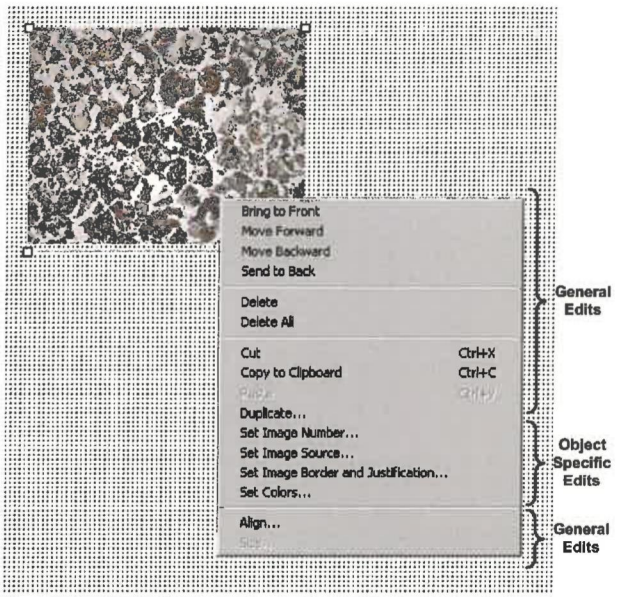
- To size objects, move the cursor either to a point on the object's border or to one of the handles. The cursor changes to a two pointed arrow
  - To resize one dimension of a box, position the cursor on a vertical or horizontal line; click and drag the side to the new size.
  - To proportionally resize a box, position the cursor on a handle; click and drag the box to the new size.
- To position objects, move the cursor over the selected object. The cursor changes to a four point arrow. Click and drag the object to a new location. In addition, if an object is selected, you can use the arrow keys to fine tune its position.

Note: When multiple objects are selected, they can be sized and positioned as a single object.

Using Right-click and Double-click Functionality

When creating a report, you can use the right-click and double-click mouse functions as editing shortcuts.

- Double clicking on a report object enables you to edit the object's attributes – e.g., the thickness of a line.
- Right clicking (Windows)/[Ctrl] clicking (Mac) on a report object calls an object specific edit menu that contains both general editing options and attribute editing options specific to that object. Right clicking on an Image object, for example, gives you a list of editing options, as shown at right.
- Right clicking (Windows)\[Ctrl] clicking (Mac) on the report grid gives you three options: **Select All** objects, **Paste** an object that you copied or cut; or **Delete All** of the objects on the grid.



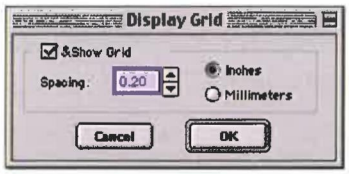
Refer to the **Defining Report Attributes** and **Editing Report Objects** sections for more details.

Defining View Options

The **View** menu provides you with eight options:

Zoom	Zoom in or out to six different sizes. From the View menu, select Zoom and enter the desired percentage.
First Page	Go to the first page in the report.
Previous Page	Go back one page in the report.
Next Page	Go forward one page in the report.
Last Page	Go to the last page in the report.

Set Display Grid Size



The report definition window includes an adjustable grid (i.e., the dots on the screen) to assist you in placing and aligning report objects on the report page. By default, the grid is turned on. To turn off or modify the grid, follow these steps:

1. From the View menu, select Set Display Grid Size.
   - To turn off the display grid, deselect the Show Grid box.
   - To modify the spacing follow steps two through four.
2. Select either inches or millimeters.
3. Enter the spacing for the display grid. The number that you enter represents the space in inches or millimeters between the dots on the grid.
   - If the measurement is **inches**, enter a number between **.04** and **10**.
   - If the measurement is in **millimeters**, enter a number between **1** and **25**.
4. Click on OK. The display grid appears.

Set Object Grid Size

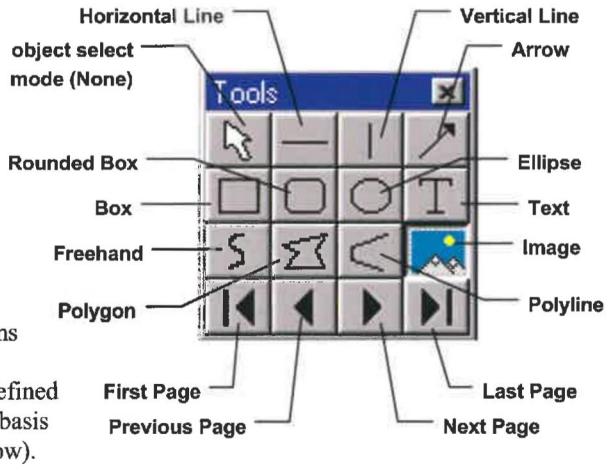
In addition to the display grid, you can define a second grid specific to report objects. The object grid controls the movement of objects as you work with them on the display grid. When you select a report object and move it, the object automatically "snaps" to the nearest line in the object grid, allowing you to more precisely align and arrange the objects on your report page.

1. From the View menu, select Set Object Grid Size.
2. If desired, check the Snap to Grid option.
3. Define the spacing for the object grid. In many cases, the object grid and the display grid will have the same spacing. In other cases, you might not want the display grid to be as finely spaced as the object grid (i.e., so the dots do not interfere with the view of the report definition window).
   - If the measurement is **inches**, enter a number between **.04** and **10**.
   - If the measurement is in **millimeters**, enter a number between **1** and **25**.

   The default spacing value for the Object Grid is the last entered value.
4. Click on OK.

Show Tools

Click on the Show Tools option to display a floating palette of navigation buttons and report objects, as shown.. Refer to the **Defining Report Objects** section of this chapter for more details.



Defining Report Attributes

All of the general style and appearance options (report attributes) can be accessed from the **Attributes** menu. Report attributes can be defined for an entire report or on an object by object basis (see the Defining Report Objects section below).

- When a report object is selected, the attribute is defined for the selected object(s) only.
- When nothing is selected, the attribute is defined for all future, relevant objects.

The following sections detail each of the report attributes and the report objects that they affect.

Colors

Define the foreground and background colors for all objects. Follow these steps:

1. From the Attributes menu, select Colors.
2. Define the **Text/Lines** color. This is the color of the text, the line, or the border around a text/image box. The default foreground color is black. To select a color click on Color.
3. The next window varies, depending on whether you are using a Windows or a Mac system.
   - Windows allows you to select from a palette of colors or enter specific HSL or RGB color values.
   - Mac OS allows you to select from a palette of colors, enter specific RGB, CMYK, HLS, or HSV values, and enter Internet specific colors.

   Define the **Background/Fill** color. This is the color that appears behind text and in boxes. Lines and images do not display a background color.

Line Thickness

Determine line thickness for the following report objects: **Line**, **Horizontal Line**, **Vertical Line**, **Arrow**, **Box**, **Rounded Box**, **Ellipse**, **Freehand**, **Polygon**, and **Polyline**.

1. From the Attributes menu, select Line Thickness.
2. Enter a Thickness value between **.05** and **10** points.
3. Click on OK.

Note: In order to define a background/fill color for a Box or a Rounded Box, a fill must be selected. Similarly, in order to define a background/fill color for a text box, the Text Style must be set to Opaque.

Defining Report Objects

All of the components of your report, including the formatting, text, images, and fields are known as objects. Using the objects, you can specify the information that your report will contain. Before discussing the individual report objects, review the following general procedure for inserting an object into a report:

1. From the Object menu, select the type of item (i.e., Lines, Connected Lines, etc…) that you want to add to the image. The cursor's functionality varies according to whether a report object type (i.e., line, text) is selected:.
   - If a report object type is selected, the cursor changers to a cross-hair icon that is used to add new report objects to the grid.
   - If **None** is selected, the cursor is a an editing tool that is used to select and position objects.
2. Use the mouse to drag the cross-hair to the location where you want to insert the object.
3. Left-click at the insertion point.
   - If you selected a line or a box, hold down the mouse button and drag the line/box to the desired size.
   - If you selected any of the other report objects, the object will appear and you will be prompted to enter the associated information (e.g., static text, the location of an image file, the database field, etc…).
4. Once it appears on screen, you can resize, reposition, and edit an object as needed.
   - **Resize** - Select (click on) the object and drag the handles in the direction of the resize.
   - **Reposition** – Left click and drag the object the new area.
   - **Edit** – Select the object and choose an option from either the Edit menu or the Attributes menu. You can also edit an object by **right clicking** (Windows)/**[Ctrl] clicking** (Mac) on it.

The following table describes the Basic program mode report objects:

None Select **None** when you want to use the cursor to edit and position the objects.

Lines

Unconstrained Line – Add a straight line annotation to the report.

Horizontal Line - Add a horizontal line annotation to the report.

Vertical Line – Add a vertical line annotation to the report.

Arrow - Add an arrow to the report. Either select Arrow from the Object menu, or click on the Tools box button. Left click on the report and drag toward the area that the arrow head will point to.

Connected Lines

Polygon – Add a closed polygon to the report. Either select Connected Lines>Polygon from the Object menu, or click on the Tools box button. Left click and release, and then drag the mouse to create a side. Left click again to start a new side. Double click to finish the polygon. The program automatically adds any additional lines needed to close the figure.

Polyline – Add an open polygon to the report. Either select Connected Lines>Polyline from the Object menu, or click on the Tools box button. Left click and release, and then drag the mouse to create a side. Left click again to start a new side. Double click to finish the polyline.

Freehand - Add a freehand line to the report.

Shapes

Rectangular Box – Add a square cornered box to the report.

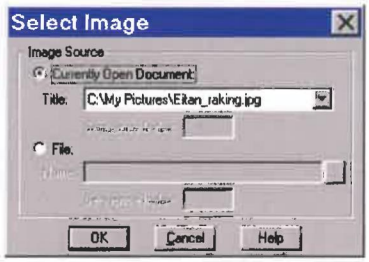
Rounded Box – Add a round cornered box to the report.

Ellipse - Add an elliptical object to the report. Ellipses are drawn inside of the rectangular area that you define. Ellipses touch the top, bottom, right and left sides of this rectangle.

Either select Shapes>Ellipse from the Object menu, or click on the Tools box button. To draw an ellipse, left click at the point where you want to position one vertex of the rectangle and drag the cursor to the point of the opposite vertex.

Image

Add an image to the report.



1. From the Object menu, select Image.
2. Left click at the point on the display grid where you want to insert the Image box, and holding down the mouse button, drag the cursor to create the desired shape and size for the image (it can also be resized/re-positioned at a later point). When you release the mouse button, the Image dialog box appears.
3. Specify the file source for the image:
   - **Currently Open Image** – Add a currently open image to the report. If you have one or more open images, SPOT lists the file name(s) and path(s) in the drop-down box. Select from the list.
   - **Image File** – Add an image from disk to the report. Specify the file name and location either by entering the path in the Name box, or clicking on the selection box to the right.
4. Click on OK. The image appears on your report grid.

Text

Text

Add text to the report.

Date

Add a date text box to the report. The current date is the default.

Time

Add a time text box to the report. The current time is the default.

User Name

Add a user name to the report. The user currently logged on to the computer appears as the default.

Editing Report Objects

Once you have created a report, you can edit the report objects as needed. There are three basic ways to edit a report object:

- **Use the Attributes menu** - The Attributes menu, as noted in the Defining Report Attributes section of this chapter, allows you to either set the attributes for adding specific types of objects, or to select existing objects and modify them as needed. Refer to the Defining Report Attributes section (above) for more details.
- **Use the Edit menu** - The Edit menu contains general editing features such as Undo, Copy, Cut, Send to Back, etc…
- **Right-click (Windows)/[Ctrl] click (Mac) on the report object** – Right/[Ctrl] clicking on a report object brings up a customized editing menu that contains both general editing commands and the attributes that can be edited for that object. Refer to the Working in the Report Mode section for more information on the right click function.

The Edit Menu

The Edit menu provides general editing features for arranging, copying, and sizing report objects. Edit menu options are active only when a report object is selected.

Undo Undo the last action. The Undo feature allows you to undo up to ten actions.

Bring to Front Bring a selected object in front of all other objects.

Move Forward Bring a selected object one layer forward.

Move Backward Send the selected object one layer back.

Send to Back Send a selected object behind all other objects.

Select All Select all of the objects in the report.

Delete Delete the selected object(s).

Delete All Delete all of the objects in the report.

Copy to Clipboard Copy the selected object(s) to the Windows clipboard. You can then paste the object(s) into:

- A new SPOT report
- A different location in the same report

This feature is particularly useful when you are creating a new report and want to use objects from an existing report.

Cut Cut the selected object(s) for pasting into a new report or to a new location in the existing report.

Paste Paste the object that you cut or copied to its new location.

Duplicate Create additional copies of a report object. To duplicate a report object, follow these steps:

1. Select the object(s) that you want to duplicate.
2. Select Duplicate from the Edit menu. The program prompts you to enter the number of duplicate copies.
3. Enter the number of copies, and click on OK.
4. The duplicate object(s) appear at the top of report window.

Add Page

Add a page to your report. When you add a page, the program prompts you with the Add Page dialog box:

1. Specify whether the page that you want to add will be inserted Before or After a specified page.
2. Specify the Page Number that the additional page will precede or follow.
3. Click on OK.

SPOT reports can have a maximum of ten pages.

Delete Page

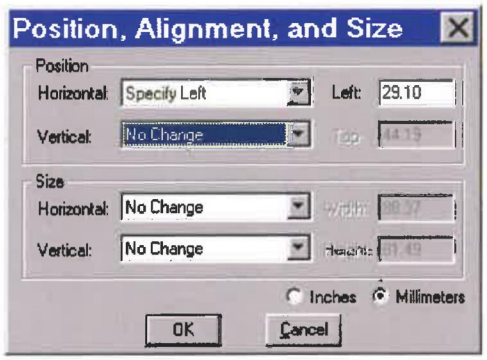
Delete a page from your report. When you select the Delete Page option, the program prompts you to specify the page number.

Add Images

Add a static image to your report.

Set Position, Alignment, and Size

The **Set Position**, **Alignment**, and **Size** option allows you to specify precise sizing and position values for single or multiple selected report and annotation objects.



The **Position** options enable you to:

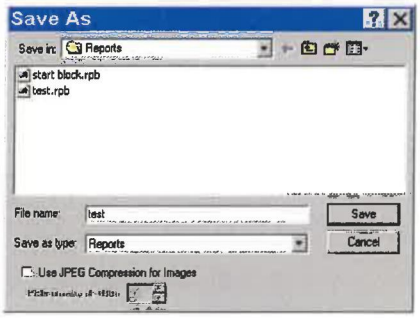
- Align top/bottom edges of multiple objects.
- Align left/right edges of multiple objects.
- Align the centers of multiple objects.
- Center an object(s) on the page.
- Enter exact values in inches or millimeters to position the object.

The **Size** options enable you to enter:

- Exact width/height in inches or millimeters for one or more objects.
- Set the width/height dimension for multiple objects the to smallest or largest object's value in inches or millimeters.

Saving Reports

1. To save a newly created report, select the **Save** option from the File menu.
2. When saving a new report (**.rpb** file type), SPOT prompts you with the default file location – i.e., **C:SpotCam\Reports** folder (Windows) or **Users\Shared\SPOT Files\Reports** (Mac) . Either save to the default location or specify a new location. If you plan to save many reports without compressing the image files, you should save them to a Jaz drive or to a network drive.

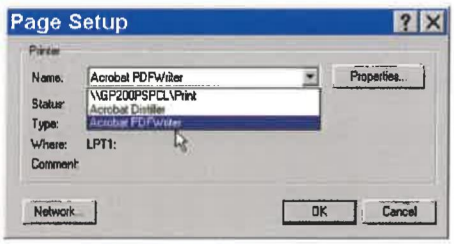


> *Note: Mac users will see the* **.rpb** *file type extension only if the* **Add Extensions to File Names on Save** *option is selected on the Preferences window .*

3. Enter a file name for the report.
4. Define the JPEG Compression for the image(s) in the report. The **Use JPEG Compression for Images** box is checked as a default. To turn off JPEG compression, deselect the box. If you are using JPEG Compression, enter a number between one and 100.
   - A value of 1 is the most compressed. File size and image resolution are decreased to the maximum extent.
   - A value of 100 is non-compressed. File size remains large and the image resolution remains high.
5. Click on Save. You can now use the SPOT program to open and view the report at a later time.

Printing and Distributing Reports

To print a report, follow these steps:



1. Open the report.
2. Select the printer. If you have Adobe Acrobat, you can create a PDF version of your report by selecting **Acrobat PDFWriter** as the printer of choice: select the Printer from the **Page Setup** dialog – i.e., **File>Printer Setup>Printer**. PDF files can be distributed to anyone with the Acrobat Reader software, which, as described below, is available from Adobe as a free download.
3. Click on Properties to define the printer's properties, if needed.
4. Specify the page range if you are not printing an entire report.
5. Specify the number of copies.
6. Click on OK.

If you intend to distribute SPOT image reports to people who do not have the SPOT program, we recommend that you purchase Adobe Acrobat. Using Acrobat, you can convert the SPOT report (.rpb) files to PDF files, which can be distributed to users who do not have the SPOT software. They can view the reports with the free Acrobat Reader software, which is included on the SPOT software CD. The latest version of the Acrobat Reader can also be downloaded from the Adobe's web site www.Adobe.com.

Image Printing

Image printing features in the SPOT basic program and the SPOT advanced program are identical. Refer to Ch. 11 – Printing Images for details.

Ch. 4 - The Advanced Program Mode

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Introduction

The **Advanced Program Mode** is an improved version of the previous SPOT software. It is designed for more advanced users who want greater control over the way images are captured and edited, and who want to make use of the image archiving database, the report generator, and the calibration mark imprinting and measuring functions.

This chapter is an introduction to the operating environment of the Advanced SPOT program mode. Detailed discussions of the features of this mode are found in chapters 5-11. This introduction is broken into three sections:

- **The Viewing and Editing Window** - Describes the primary window for all image capture and editing functions.
- **Working With Image Files** - Discusses file save and open features.
- **Setup** - Describes the initial Setup options.

The Viewing and Editing Window

When you open or capture an image, it appears in the main viewing and editing window. All of the image save, view, edit, and annotation functions are accessed from this window, which consists of three areas:

- Menus
- The Toolbar
- The Status Bar

Menus

The menu bar in the Advanced program varies according to whether an image is open. The table below briefly describes each menu, indicates whether an image must be open in order to see it, and provides a reference to the chapter that discusses the menu options in more detail:

Menu	Functions	Image Must be Open?	Ch. Ref.
File	Open, save, and print files. Create and run reports.	No	4, 10
Database	Create, access, and modify SPOT image databases.	No	9
Edit	Edit and annotate images.	Yes	8
Sequence	Viewing/play options for image sequences.	Yes	8
View	Viewing options for captured images	Yes	4
Camera	Initialize the camera; view live preview images; capture images; perform white balances.	No	6, 7
Setup	Define image setups, image capture preferences, calibration setups.	No	5
Window	Standard Windows display options.	Yes	4
Help	Information about the SPOT software and an on-line help system.	No	

The Toolbar

The Toolbar provides shortcut buttons to frequently used menu items. Dragging the cursor over the Toolbar displays information about each button.

- The name of the button (and the associated menu option) displays on the toolbar.
- The function that the button performs displays on the left of the status bar.

Refer to Appendix C: Keyboard and Toolbar Button Commands for a complete listing of command equivalents in the SPOT program.

Like the menus, the Toolbar options vary according to whether an image is open, and what you are doing. The toolbar displays the options necessary in each of the following situations:

- When you first open the SPOT program…
- After you have captured or opened an image…

When You First Open the SPOT Program...

When you first open the program, the toolbar displays only the image capture options:

Toolbar Button		Description
	Live Image	Show/hide live image window
	Get Image (exposure from setup)	Take a picture
	Get Image (last used exposure)	Take a picture using the last used exposure
	Compute White Balance Values	Compute RGB white-balance ratios
	Full Screen	Toggle full screen mode
	Search Database	Search the database

(The Search Database option is active only when a database is open.)

Insight B/W	If you are using an Insight B/W camera, the Compute White Balance Values option is inactive.

Refer to Ch. 6 – Working with Live Images, Ch. 7 - Taking a Picture, and Ch. 8 - Viewing, Editing, and Annotating Images for more details.

After You Have Captured or Opened an Image...

After you have either captured a new image or opened an existing image, the toolbar expands to include the file view, save, and print options:

Toolbar Button		Description
	Zoom	Turn on/off zoom mode
	Show Pan Window	Show/hide the pan window (useful for navigating when zoomed)
	Save	Save the image
	Save As	Save the image to disk with a new name and/or type
	Save to Database (Windows)	Save the image to the database *(this option is active only when a database is open)*

(cont.)

Reload	Reload	Reload the image from disk
	Print	Print the image

Refer to Ch. 8 – Viewing, Editing, and Annotating Images and Ch. 11 - Printing Images, for more details.

The Status Bar

The status bar, which appears at the bottom of your screen, displays information about the image that is on screen. The following is an illustration of the view status bar, as it appears for an open image:

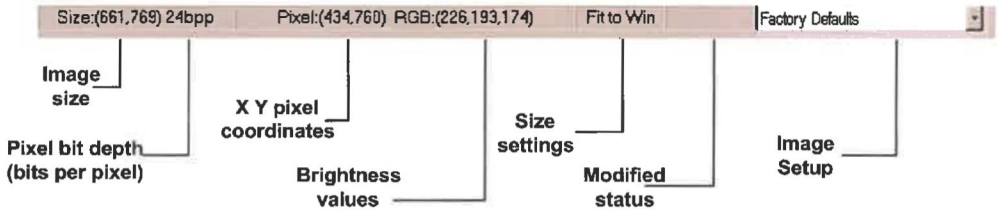


Figure 4- 1: Advanced Mode Status Bar

Each of the call-outs is described below.

- **Image size** – The number of columns **x** the number of rows of pixels in the image.
- **Pixel bit depth (bits per pixel)** - The pixel bit depth of the image. There are four options:
  - 8 bpp (monochrome) – Insight B/W
  - 12 bpp (monochrome) – Insight B/W
  - 24 bpp (RGB color) – Insight Color
  - 36 bpp (RGB color) - Insight Color

  For a detailed discussion of pixel bit depth, refer to Ch. 5 - Preparing to Take a Picture.
- **X Y pixel coordinates** - As you move your cursor over an image, the status bar displays the x y coordinates of the cursor in pixels. For example, the values for the upper left corner pixel would be (0,0).
- **Brightness values** - As you move your cursor over an image, the status bar indicates the pixel brightness values at the indicated x y pixel coordinates. The values depend on the pixel bit depth (see Ch. 5 – Preparing to Take a Picture) for the image. For example, if a color image is open, the status bar displays separate red, green, and blue brightness values [e.g., RGB: (162, 134, 127)]. If a monochrome image is open, the status bar displays only one brightness value [e.g., (176)].
- **Size Settings** - In the illustration above, the view status bar displays **Fit to Win** (Fit to Window), the default image capture setting.
  - If the Fit to Window option has been deselected, this area displays the word **Normal**.
  - If you are in zoom mode, this area displays the magnification level (e.g., **Mag:x1**, **Mag:x1/4** etc…).
- **Modified Status** - If you open an image file or take a picture, the section to the right of the magnification setting remains blank until you modify that image. Once that image has been modified, the word **Modified** appears here.

- **Image Setup** - The far right end of the view status bar displays the currently active Image Setup (e.g., Factory Defaults). This is the image setup that the program uses to capture images. From this part of the view status bar, you can:
  - Change the currently active image setup.
  - Modify an existing image setup.
  - Add a new image setup to the list.

Refer to Ch. 5 - Preparing to Take a Picture, for more information on image setups.

Working With Image Files

The File menu contains four categories of options:

- Opening files
- Reporting
- Saving, closing, and deleting files
- Printing

The following table describes these options in the order that they appear on the menu.

Open Image File

Open image files of a specified format (i.e., JPEG, GIF, BMP, etc.). In addition to using this menu option, you can open images by dragging the image file from the Windows Explorer onto the SPOT program window.

Show Image File Thumbnails

Display thumbnail previews of specified files. From the thumbnail display window, there are a variety of options. To view image file thumbnails, follow these steps:

1. From the File menu, select the Show Image File Thumbnails option. The Thumbnail File Spec window displays all of the image files in the directory.
2. Navigate to the directory for the image files that you want to display.
3. Use the **Files of type** drop down box, to specify the file type.
4. Select the file or files for which you want to display thumbnails. If you do not select a file or files, the program displays thumbnails for all files of the specified type in the directory.
5. Click on Open. The Thumbnails preview window appears. For each thumbnail, you can:
   - ***Double-click*** (Mac and Windows) on a thumbnail to open an image for viewing or modification.
   - ***Right-click*** **(Windows)/[Ctr] Click (Mac)** on a thumbnail to display a list of options. The right-click menu enables you to perform a variety of actions directly from the thumbnail window:
     - **Select Image.**
     - **Open Image.**
     - **Delete Image.**
     - **Print image.**
     - **Move/Rename Image File**
     - **Copy Image File**
     - **Add Image to Image sequence** (see below)
     - **Add Image to Report Template** (see below)
     - **Select All Images** in the thumbnail view. When you use this option, the right-click menu expands to include the above options for multiple images
6. Click and drag with the mouse to select two or more images.

Open Image Sequence File Open an image sequence file. An image sequence file is a single TIFF format file that contains multiple images.

- Refer to Ch. 7 – Taking a Picture for information on capturing an image sequence file.
- Refer to Ch. 8 – Viewing, Editing, and Annotating Images for information on viewing and editing image sequences.

Open Database Image Open images from a SPOT database. The database must be open in order to use this feature. Refer to Ch. 9 – Using SPOT Databases for details on working with databases and database images.

New Image Sequence Create a new image sequence file. This option enables you to specify the first image in a new image sequence. This image can come from an open image, an image on disk (from file), or a SPOT database. Subsequent images can be added to the sequence in one of two ways:

- Via the **Add Image to Image Sequence** File menu option (see below). This option allows you either to add an open, non-sequence image to a sequence or to create a new image sequence from the image.
- Via the **Sequence** menu options. Here you can add any image to any point in a sequence. Refer to **Working With Image Sequences** in Ch. 8 for more details.

New Report Create a new Advanced mode report. Refer to Ch. 10 – Reporting for details.

Open Report Open an Advanced mode report. Refer to Ch. 10 – Reporting for details.

Run Report Run a report. Reports can be run in three ways - previewed, printed, or saved to file. Refer to Ch. 10 – Reporting for details.

Reload Reload the image from the hard drive (or other media). All changes made since opening the image file are lost. This option displays only when a saved image is open.

Save Save a previously unsaved image to new file, or update the changes to an existing file. This option appears only when an image is open.

Save As Save an image to a new file and/or file location. This option appears only when an image is open.

Save All Save all open image files. The program prompts you for a name, file location, and image file format for each image.

Close Close an image. The program prompts you to specify a location to save the file. This option appears only when an image is open.

Move/Rename Move and/or rename an image file. This option differs from the Save As option in that it *does not* retain a copy of the original file in the original location: using Move/Rename is comparable to cutting and pasting a file.

Delete Delete an open image. The program prompts you to verify the deletion.

Export Movie File (Windows)

Export a SPOT image sequence file to AVI format. Follow these steps:

1. Open a SPOT image sequence. Refer to Ch. 7 – Taking a Picture, for details on capturing image sequences.
2. Select **File>Export Movie File**. The **Export Movie File** appears.
3. Enter the **File Name** for the avi file.
4. Specify the location where you want to save the avi file.
5. Specify which images in the sequence that you want to **Export**. Select either:
   - **All Images** – the avi file will contain all of the images in the sequence.
   - **Specified Images** - the avi file will contain only the images that you specify (i.e., below the radio button).
6. Specify the **Frame Rate** in frames per second.
7. Specify the **Compression Settings**. When you click on the **Compression Settings** button, the **Video Compression** dialog displays the compression application(s) available on your computer. Because each computer will have different installed applications, the Video Compression options will vary.
8. After you have entered all of the desired settings, click on **Save**. The program displays a status message as it exports the file.

Add Image to Image Sequence

Add an open, non-sequence image to an open image sequence, or create a new image sequence from an open image. To use this option, the non-sequence image must be the active window and the image sequence must be open in the background.

When you click on **Add Image to Image Sequence**, SPOT prompts you to specify either the **Image Sequence** or a new image sequence. If more than one image sequence is open, select the sequence from the drop-down list.

Add Image to Report Template

Add a selected open image to an open SPOT report template. When you select this option, SPOT prompts you to select from a list of report templates. If you have not defined a report template, SPOT automatically creates a new report for you and inserts the image as a **Static Image** object. Refer to Ch. 10 – Reporting for details on working with SPOT reports.

Print

Print an open image. Refer to Ch. 11 – Printing Images for details.

Print Setup

Define the page setup parameters. Refer to Ch. 11 - Printing Images for details.

Exit (Windows) Quit (Mac)

Exit the SPOT program.

Setup

The Setup menu options allow you to define operational standards used for image capture and editing. Refer to the sections below for descriptions of each option.

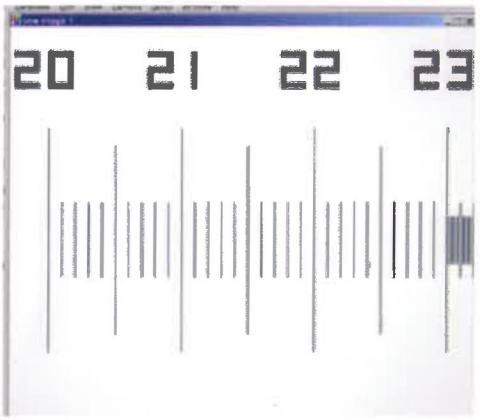
Image Setups

In the SPOT software, an image setup serves two purposes:

- It defines the way a picture will be taken.
- It defines the way the program will automatically modify a picture after it is taken.

Pre-configured image setups for standard microscope techniques (e.g., brightfield, fluorescence, etc.) help novice users to take good pictures the first time. Experienced users can modify these pre-configured setups as needed. Refer to Ch. 5 - Preparing to Take a Picture for details.

Calibration Setups



With the SPOT program, you can use a stage micrometer to calibrate each objective on your microscope. Once calibrated, you can use the SPOT program to perform basic measurements on an image. Refer to Ch. 8, Viewing, Editing, and Annotating Images for more information on using the measurement functions.

To calibrate an objective, follow these steps:

1. Place the stage micrometer under the objective.
2. Adjust the microscope to display as much of the stage micrometer as possible. You can use the Live Image option, as described in Ch. 6 – Using Live Images, to help position the micrometer
3. Focus on it. Use the Live Image option.
4. Capture an image of the stage micrometer. Refer to Ch. 7 – Taking a Picture for details.
5. From the Setup menu, select Calibration Setups. The Calibration Setups window appears (see right).

6. Click on Add. The Calibration Setup window appears.
7. Enter the name of the calibration setup. Typically this corresponds to the magnification of the objective (e.g., 4x).

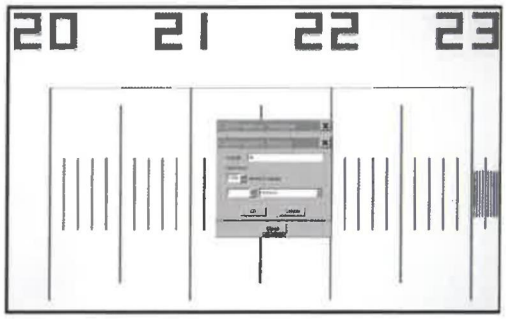
8. Using the mouse, draw the calibration line along the length of the stage micrometer image. When you draw the line, keep these guidelines in mind:
   - Keep the line as straight as possible.
   - Make the line as long as possible.
   - Use the largest unit of measurement that the micrometer allows for.

To draw the line, follow these steps:

a. Position the crosshair cursor over the stage micrometer mark that represents the start point of your calibration line. In the illustration below, this is the 20 mm mark.

b. Left click on the mouse.

c. Position the crosshair cursor at the stage micrometer mark that represents the end point of your calibration line. In the illustration below, this is the 23 mm mark.



d. Left click on the mouse again. The length of the line in pixels appears on the Calibration Setup window. To re-draw a line, double click anywhere on the image. This clears the previously drawn calibration line from the screen.

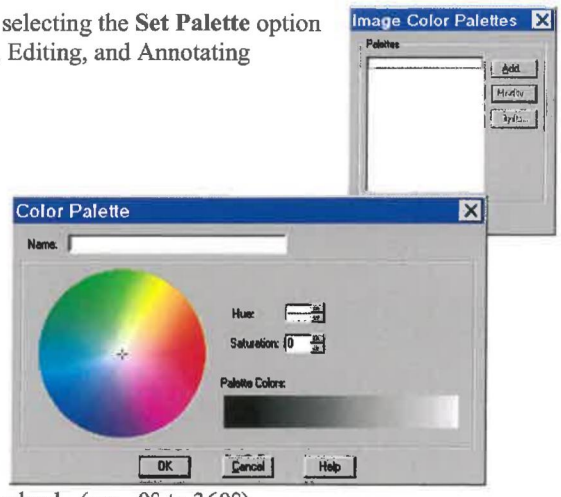
9. Enter the length and units of the line that you drew (e.g., 3 mm).

10. Click on OK. The 4x objective is now calibrated to perform measurements. Repeat steps one through ten for each objective.

Palettes

The Palettes option on the Setup menu allows you to define new palettes and edit existing palettes. SPOT color palettes allow you to apply specific hue and saturation values to monochrome (8 bit or 12 bit) images. Palettes are useful in fluorescence applications where a sample is fluorescing at a single wavelength, and you want the acquired image to have the correct color. For such a case, you could create a palette to represent the color of the fluorescent dye and then apply that palette to the captured image. There are two ways to apply a palette to an image:

- You can associate a palette with an Image Setup, as discussed in Ch. 5 - Preparing to Take a Picture.
- You can apply a palette to a captured image by selecting the **Set Palette** option from the **Edit** menu. Refer to Ch. 8 – Viewing, Editing, and Annotating Images for more details.



To define a new palette, follow these steps:

1. From the **Setup** menu, select **Palettes**.
2. Click on **Add**. The Color Palette dialog appears as shown here:
3. Either use the **Hue** and **Saturation** selection lists or move the cross-hairs on the color wheel to enter the values
   - **Hue** is the designation for the actual color, as distinguished from others in the color spectrum. Hue is derived from the color wheel and is expressed in the color's angular location (in degrees) on the wheel. (e.g., 0° to 360°).
   - **Saturation** is the proportion of perceived pure hue in the color. Saturation (in the SPOT software) is measured on a numerical scale of 0 to 100, where zero is pure gray and 100 is pure hue (e.g., day glow).
4. Click on **OK**.

Image Auto-Save (Windows)

Use the **Image Auto-Save** feature to automatically save images as part of the capture process. To enable SPOT's auto-save feature, select **Setup>Image Auto-Save**, and check the **Auto-Save Images** box, as shown at right. You can then specify the auto-save options, as described in the table below.

File Name Prefix This prefix appears before the number of each image capture in the sequence. To enter the File Name Prefix, you can:

- Type only the name (e.g., **Cell**) in the box. The program automatically saves the image to the last used directory. If you specify the prefix only, you cannot see exactly where the files are being saved. Thus, to avoid having to search for your files, you should verify the location of the last save.
- Type the full path and file name (e.g., **C:\Spotcam\T-cells**) in the box to specify the location of the image. When entering the file path and name, you can also specify a new directory simply by entering it in the path (e.g., C:\Spotcam**Cells**\T-Cells).
- Click on the browse button to select a new file location and file name (prefix). All of the image files are saved with the prefix that you enter. Thus, if your Suffix Begin Number (see below) is one, the file name for the image would be **T-Cells0001**.

Format Select the file format for the sequence of images.

- For individual files, there are six options: **Windows Bitmap**, **JPEG**, **Mac PICT**, **TIFF Uncompressed TIFF JPEG**, or **JPEG2000**.
- For image sequences there are two options: **TIFF Uncompressed** or **TIFF JPEG**.

Suffix Begin Number Select the beginning number for image auto-save.
The Begin Number automatically defaults to the first number that follows the previous sequence of images. So, if you captured a sequence of 10 images, 11 would appear as the Begin Number when you next opened the Get Sequential Images window.

The program prefaces numbers with enough zeros to create a four character suffix. For example, if you have 35 images, the first image suffix will be **0001** and the last image suffix will be **0035**. This enables Windows to correctly order images, so that Growth_Sequence0002 appears before Growth_Sequence1000, and not the other way around.

JPEG Quality (1-100) JPEG quality is the level of compression for JPEG images: this option is available only when JPEG or TIFF JPEG is selected as the image file format. Enter an integer between 1 and 100 to set the JPEG quality for images. A value of one is the most compressed (and lowest image quality), while a value of 100 is the least compressed (and highest image quality).

Compression (JPEG2000) When the JPEG2000 file format is selected, the Image Auto-Save Setup dialog displays compression options:

- **Lossless** – Information is not lost when the image is compressed.
- **Lossy** – Information is lost according to the ratio that you enter: the higher the ratio that you enter, the smaller the file size and the lower the quality of the image.

Save Thumbnails Save thumbnails of each image capture. This option is available only when JPEG, TIFF JPEG, or TIFF Uncompressed is selected as the image file format.

File Name Conflict Action Determine how the program will respond if it finds an image file with the same name as an image from the current session. Select one of the following options:

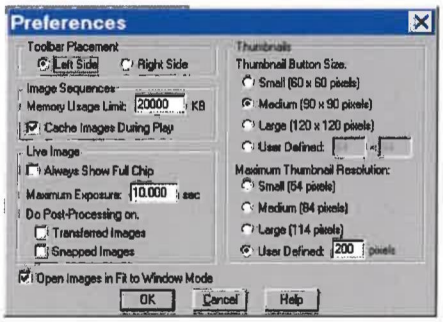
- **Overwrite Existing File** – The program overwrites the existing file with the new image capture.
- **Display Warning** – The program displays a warning message indicating that a file of the same name already exists, and stops the image capture.

Image Display Determine how image captures will be displayed. Select one of the following options:

- **Display Each Image in a New Window** – The program opens a window for each new image capture. This option works well if you plan to capture a limited number of images in the session. If you plan to capture many images, however, displaying numerous windows uses up memory and could interfere with use of other programs.
- **Display Only Current Image** – The program sequentially displays each image in the same window. Each image displays only until the next image is captured. This is useful when you have numerous captures where you do not want to be concerned with running out of memory.
- **Don't Display Images** – The program automatically saves all of the image files to the specified location without displaying them.

Preferences

The SPOT program allows you to set basic operating preferences. Select **Setup>Preferences**. The **Preferences** window appears, as illustrated at right. Each option is described in the table below.



Toolbar Placement Select the screen position for the Toolbar Placement.

Image Sequences

Memory Usage Limit

Specify how much memory is allocated for image acquisition. Note that the entry parameters are system dependent.

Cache Images During Play

When "playing" an image sequence (see Ch. 8), the SPOT program allows you to select how it loads the displayed images:

- *If **Cache Images During Play** is selected…* The program pre-loads as many images from the sequence as your computer's RAM allows. This enables a faster display of the image sequence. But, if you have a sequence with many images, the size of the combined images could exceed the RAM, and in this case, the SPOT program will load as much of the sequence as possible, play that part of the sequence, and then pause to load the remaining images.
- *If **Cache Images During Play** is not selected…* The program loads each image in the sequence individually. In cases where you have a large sequence with many files, this allows you to work without being restricted by RAM limitations. The display of images will be somewhat slower than a display where images are cached.

Live Image

Do Post Processing On

Specify whether to apply the **Post Processing** options for the current Image Setup to images captured from the live image view.

- **Transferred Images**
  - *If the **Transferred Images** box is selected,* the Transfer option applies the **Noise Filter**, **Color Enhancement**, and **Image Size Double** options as specified for the current Image Setup.
  - *If the **Transferred Images** box is unselected,* the **Transfer** option ignores the Post Processing options for the current Image Setup, with one exception: if the **Chip Defect Correction** option is selected for the current image setup (SPOT and RT cameras only) , the corrections will appear in the image capture, regardless of the Transferred Images preference.
- **Snapped Images**
  - *If the **Snapped Images** box is selected,* the Snap option uses all of the Post Processing options from the current Image Setup, except for Background Subtraction and Flatfield Correction.
  - *If the **Snapped Images** box is unselected,* the Snap option ignores the Post Processing options for the current Image Setup, with one exception: if the **Chip Defect Correction** option is selected for the current image setup (RT and SPOT cameras only), the corrections will appear in the image capture, regardless of the Snapped Images preference.

Thumbnails

Thumbnail Button Sizing

Specify the size of thumbnails for the Show Image File Thumbnails option and database search results

Select from one of the following options:

- **Small (60 x 60 pixels)**
- **Medium (90 x 90 pixels)**
- **Large (120 x 120 pixels)**
- **User Defined**
  Minimum width and height = 20 pixels
  Maximum width and height = 200 pixels

Maximum Thumbnail Resolution

Specify the resolution of thumbnails for the Show Image File Thumbnails option and database search results

Select from one of the following options:

- **Small (54 pixels)**
- **Medium (84 pixels)**
- **Large (114 pixels)**
- **User Defined**
  Minimum resolution = 20 pixels
  Maximum resolution = 200 pixels

Open Images in Fit to Window Mode

If this option is selected, the SPOT program automatically sizes images to fit the available screen space when you open or capture an image. Thus, you can immediately see the entire image without having to zoom out. This is especially useful with smaller monitors.

Add Extensions to File Names on Save (Mac)

When selected, the **Add Extensions to File Names on Save** option automatically appends the file type extension (e.g., JPG, tif, bmp, etc...) to your image file. This enables cross platform file sharing, because Windows based programs require a file type extension, while Mac based programs do not. Once the file type extension is added, Windows based programs can recognize the Mac file.

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Introduction: What is an Image Setup?

An image setup serves two crucial purposes:

- It defines the way a picture will be taken.
- It defines the way a picture will be modified after it is taken.

Image setups are defined to optimize the picture taking process for different types of images or microscopy techniques. They can be created, saved, and recalled as needed.

Accessing the Image Setup Window

To define a new image setup or to edit an existing setup, follow these steps:

1. From the Setup menu, select the Image Setups option. The Image Setups window appears. Either double-click on a setup to open it, or select one of the following options:

   – **Add** - To create a new image setup, click on Add. The New Image Setup dialog box appears with a list of templates (image types) that serve as the basis for the new image setup.

   – **Modify** - To edit an existing image setup, select a setup from the list, and click on Modify. The Image Setup window appears.

   – **Delete** - To delete an image setup, select a setup from the list and click on Delete.

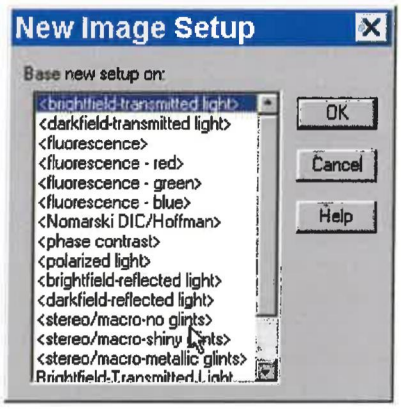
   – **Current** – To make a setup the active setup, select the setup from the list and click on Current.

   – **Close** - To exit the dialog box, click on Close.

Shortcuts: You can quickly access the Image Setup window by right clicking the drop-down box in the bottom right corner of your screen. The box displays the name of the current (active) Image Setup.

- To **change the current (active) image setup**, left click on the tab and select the new Image Setup.
- To **define a new image setup** or to **modify an existing image setup**, right click on the box, and select Add.
- To **modify the current image setup**, right click on the box and select modify.
- To **access the current image setup**, press **[F12]**.

If you select the Add option (i.e. to create a new image setup), the New Image Setup window appears. The pre-defined templates (image types) are indicated by brackets < >, while user-defined setups appear without brackets:



Insight B/W	Insight B/W camera users will not see the fluorescence – red, fluorescence – green, and fluorescence – blue Image Type options.

2. **Select an image type from the list.**
The 14 pre-defined image types (in addition to the Factory Defaults setting) correspond to commonly used microscopy techniques. To define a new image setup, you must also select an image type as a template for defining the new setup. The image type provides a baseline set of values for that setup, according to the microscopy technique you are using. The following table describes SPOT's pre-defined image types:

Image Type	Description
Brightfield-transmitted light	Stained specimens on glass slides using transmitted light microscopes.
Darkfield-transmitted light	Bright specimens on dark backgrounds using transmitted light darkfield technique.
Fluorescence	Fluorescence specimens not fluorescing in a single red, green, or blue primary color. The camera expose images using all three colors.
Fluorescence – red	Fluorescence specimens fluorescing in red. Exposure time is reduced because only one color is exposed.
Fluorescence – green	Fluorescence specimens fluorescing in green. Exposure time is reduced because only one color is exposed.
Fluorescence – blue	Fluorescence specimens fluorescing in blue. Exposure time is reduced because only one color is exposed.
Nomarksi DIC/Hoffman	Specimens being viewed using Nomarski DIC or Hoffman techniques.
Phase contrast	Specimens being viewed using the phase contrast technique.
Polarized light	Specimens being viewed with a polarized light microscope.
Brightfield-reflected light	Polished metallurgical specimens and semi-conductors using a microscope with a vertical illuminator.
Darkfield-reflected light	Bright areas of specimens being viewed with darkfield, reflected light techniques.
Stereo/macro–no glints	Matte specimens without bright glints.
Stereo/macro–shiny glints	Shiny specimens with bright glints.
Stereo/macro-metallic glints	Metallic specimens with intense glints.

3. **Click on OK.**
   The Image Setup window appears.

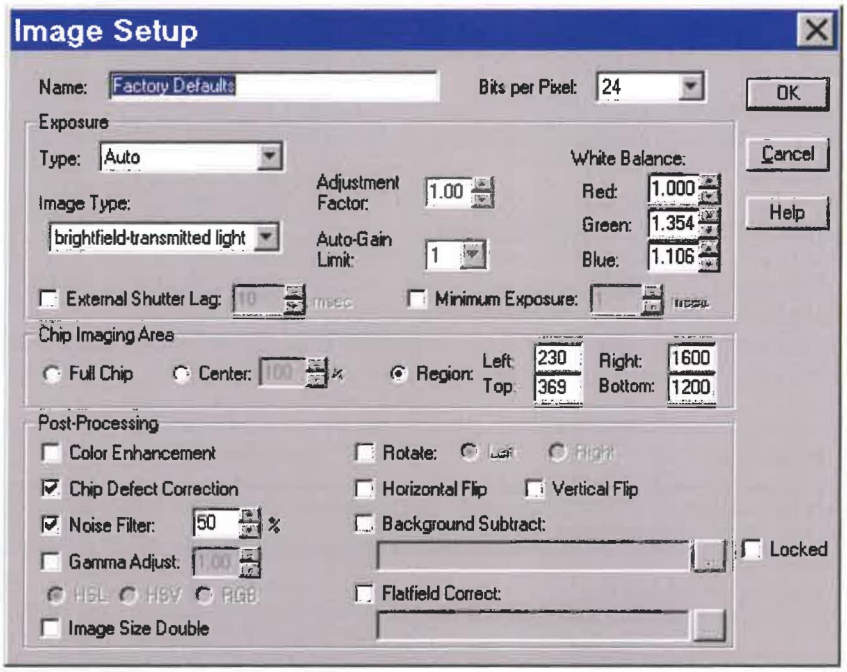


Image Setup Options

The Image Setup window consists of five sections:

- Name
- Acquired Image - Bits per Pixel and Palette (Insight B/W only)
- Exposure
- Chip Imaging Area
- Post Processing

Name

Use this box to enter the name of a new image setup, or to modify the name of an existing setup. If you modify the name of an existing setup, the program prompts you (when you click OK) to save this as a new setup or overwrite the original setup.

Acquired Image

The Acquired Image section options enable you to define the characteristics of captured images. This includes:

- Bits per Pixel
- Palette (Insight B/W only)

Bits per Pixel

Bits per pixel or *Pixel Bit Depth* is the number of *bits per pixel* (bpp) that the camera uses to create an image. The SPOT program provides different options, depending on whether you have a color or monochrome Insight camera:

- **Insight Color - 24 bpp** and **36 bpp**.
- **Insight B/W - 8 bpp** and **12 bpp**.

Note: Insight Color users can use the Change Pixel Bit Depth *option to convert the pixel bit depth for a captured image to a monochrome setting.*

For Insight Color users, the 24 bpp RGB color setting is the most common selection because it is an industry standard color format that is used by Windows and most printers. Most monitors cannot display more than 24 bpp.

Select a Pixel Bit Depth by clicking on the Bits per Pixel drop-down box. In addition to specifying the bits per pixel of the image, your selection determines the Gain and Auto-Gain Limit choices (see appropriate sections in this chapter), as illustrated in the table below:

Bits Per Pixel	Camera	Available Gain/Auto-Gain Limit Options
8	B/W	1, 2, 4, 8, 16
12	B/W	1,2
24	Color	1, 2, 4, 8, 16
36	Color	1,2

The following table lists the available image depth options and describes some of the potential uses, advantages, and disadvantages associated with each setting.

Pixel Bit Depth Settings	Characteristics
8 bpp (monochrome) ***Insight B/W***	The 8 bit setting is often used in cases where 256 gray levels are adequate. The lower number of bits per pixel results in smaller, more manageable file sizes. *A typical 8 bpp image is… 1.83 Mb*
12 bpp (monochrome) ***Insight B/W***	The greater number of bits per pixel in the 12 bit setting provides users with 4096 gray levels. This provides a more accurate means of measuring brightness in a monochrome image, and helps to reduce *banding* problems that often appear when an image is modified. *A typical 12 bpp image is… 2.75 Mb*
24 bpp (RGB color) ***Insight Color***	The 24 bit setting provides 8 bits per color, per pixel (i.e., 8 x 3 = 24). As noted, 24 bpp is the most common choice because it is an industry standard in terms of printing, the Windows operating system, and monitor display. *A typical 24 bpp image is… 5.49 Mb*
36 bpp (RGB color) ***Insight Color***	The 36 bit setting provides 12 bits per color, per pixel (i.e., 12 x 3 = 36). 36 bpp is used in cases where images are heavily modified. The 36 bit setting provides users with 4096 brightness levels per color and thereby reduces banding problems when images are stretched. *A typical 36 bpp image is… 8.24 Mb*

Palette (Insight B/W)

SPOT color palettes allow you to apply specific hue and saturation values to monochrome (8 bit or 12 bit) images. Palettes are useful in fluorescence applications where a sample is fluorescing at a single wavelength, and you want the acquired image to have the correct color. For such a case, you could create a palette to represent the color of the fluorescent dye and then apply that palette to the captured image. The **Palette** option on the Image Setup window allows you to automatically apply a palette to a monochrome image capture.

Note: Applying a color palette to an image capture does not affect image data.

To apply a palette, select one from the drop down list. There are four pre-defined palettes: **Red**, **Green**, **Blue**, and **Grayscale**. Because the **Palettes** option applies only to monochrome images, it is available only when either an 8 bpp or 12 bpp Pixel Bit Depth is selected. You can define custom palettes using the **Palettes** option on the **Setup** men. Refer to Ch. 4 – The Advanced Program Mode for more information.

Exposure

The Exposure options tell the camera what settings to use when taking a picture. Carefully considered definition of these settings helps to ensure that your images come out as planned.

Note: The available exposure options vary according to the selected Pixel Bit Depth.

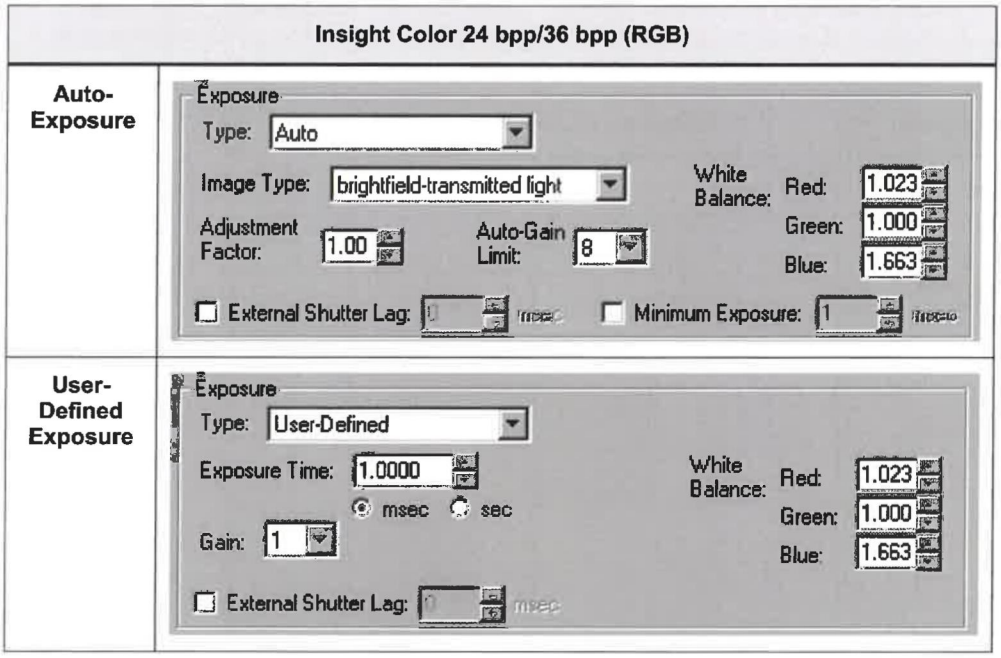
Type: Auto vs. User-Defined Exposure

With the SPOT camera, you can either allow the camera to calculate exposure time (Auto), or you can calculate the exposure on your own (User – Defined).

- When you select **Auto**, the camera samples light levels (as the initial part of the image capture) and uses the values to calculate the final exposure time.
- When you select **User – Defined**, you have three options:
  - You can type in values for the red, green, and blue exposure times.
  - You can use the **Compute Exposure** option to calculate and insert the exposure times (and gain) for you.
  - You can use the **Recall Previous Exposure** option to recall and insert the exposure times and gain used for the last picture.

The following table compares the available exposure options for automated and user defined exposures for the Insight Color camera.

Note: The Insight B/W camera options are identical to those pictured below, with two exceptions: Insight B/W cameras feature a binning option, and there are no White Balance values.



Note: If you select 36 bpp as the pixel bit depth, Gain values/Auto-Gain Limits of 16 are not available.

Binning (Insight B/W)

Binning is a pre-exposure option that tells the camera to combine adjacent pixels on the CCD chip. The camera treats each set of combined pixels as one large pixel. The large pixels accumulate electrons faster and are therefore more sensitive to light. Binning has three effects on image processing:

- Light sensitivity is increased.
- Download time is decreased.
- Resolution is decreased.

Binning is particularly helpful when you want to drastically reduce exposure and download times for dim images and you don't mind a decreased resolution. This gives you higher frame rates and less noisy images. The SPOT camera offers four binning options, as illustrated below:

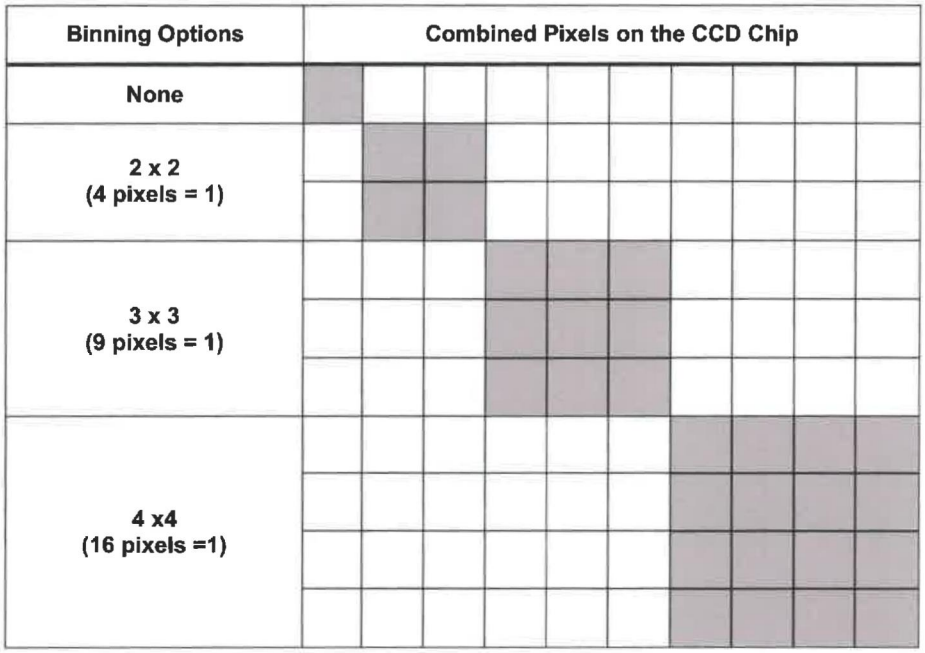


Figure 5- 1: Binning Options

The default Binning option is set to None.

Image Type (Auto-Exposure)

When using Auto-Exposure, the SPOT software provides 11 image type settings that correspond to various types of microscopy. The image type that you enter when creating a new setup appears on the Image Setup window. You can enter a new Image Type from the Image Setup window. Refer to the Accessing the Image Setup Window section of this chapter for descriptions of the different image types.

Auto-Gain Limit (Auto-Exposure)

Normally an auto exposure routine in a digital camera sets the exposure time so that the brightest pixel on the CCD chip is completely "filled" with electrons, or is "full well". This simple technique works well for bright images, but as an image gets dimmer, exposure times can get uncomfortably long. With the SPOT camera you can shorten exposure times by filling the brightest pixel on the CCD to less than full well, and then using this lower level as *full scale*. The inverse fraction of full well that the brightest pixel on the CCD is filled to is known as the *gain*. For example, filling a pixel to half of full well cuts the exposure time in half. Since the exposure time is cut in half, we call this a gain of 2.

The **Auto-Gain Limit** feature allows you to set an upper limit on the gain options that will be available during the Auto-Exposure process.

Note: If you select 36 bpp as the pixel bit depth, Gain values/Auto-Gain Limits of 16 are not available.

For example, with an Auto-Gain Limit of 16 the camera can "choose" from the full range of gain options. However, if you find that gains of 8 or 16 are too noisy for your images, you can prevent the camera from selecting these options by setting the Auto-Gain Limit to four. When the Auto-Gain Limit option is set to four, the camera is limited to the lower three gain options: 1, 2, or 4. The following table illustrates the five levels of gain, the fraction of full well that the brightest pixel is filled to for each level, and a sample exposure time for each level:

Gain	Fraction of Full Well	Exposure Time (sample)
1	1	80 seconds
2	½	40 seconds
4	¼	20 seconds
8	1/8	10 seconds
16	1/16	5 seconds

Table 5- 1: Gain Settings

Note: If you select 36 bpp as the pixel bit depth, Gain values/Auto-Gain Limits of 16 are not available.

Higher gain levels for dim images significantly reduce exposure time. For each doubling of gain number, exposure time is halved. Thus, an image with an exposure time of 5 minutes at full scale (a gain of 1) would take only 1.25 minutes at a gain of 4. Unfortunately, background noise increases proportionally to the gain. Thus, the shortened exposure time would result in an image with four times the amount of background noise.

The following table illustrates the relationship between gain, exposure time, and background noise:

Auto-Gain Limit	Possible Auto – Gain Settings	Exposure Time (dim images)	Background Noise (dim images)
1	1	Longest	Lowest
2	1, 2	↑	↓
4	1, 2, 4	↑	↓
8	1, 2, 4, 8	↑	↓
16	1, 2, 4, 8, 16	Shortest	Highest

Table 5- 2: Relationship Between Gain, Exposure Time and Background Noise

To set the Auto-Gain Limit, follow these steps:

1. Ensure that the camera is set to use the Auto-Exposure option.
2. Click on the Auto-Gain Limit select list, and select the number that you want to limit the gain to.

For more information on the concept of gain, refer to Appendix A: Electronic Imaging Theory and the SPOT Insight Camera.

Adjustment Factor (Auto-Exposure)

Use the Adjustment Factor option to modify the automated exposure settings determined by the SPOT camera. The Adjustment Factor defaults to a setting of 1 (i.e., 1x the exposure times determined by the Auto-Exposure setting). The Adjustment Factor is used to deliberately over-expose or under-expose an image.

- If an image that you captured (using the default Auto-Exposure settings) comes out too bright or too dark, you can re-capture the image using a different Adjustment Factor to compensate for the excess brightness or darkness.
- If you use the SPOT camera to take pictures of printed circuit boards or other metallic objects, the Adjustment Factor allows you to over-expose the image in order to burn out the glints that appear when capturing images of metallic objects. Over-exposing the glints is necessary to correctly expose the non-glinted areas.

To set the adjustment factor, enter a number between .25 and 32.

- Values **greater than 1** *overexpose* the image.
- Values **less than 1** *underexpose* the image.

CAUTION!

To avoid blooming problems in cases where you have entered an Adjustment Factor of 2 or higher, ensure that the Auto-Gain Limit is set to 16.

White Balance (Insight Color)

White balance is the ratio of red, green, and blue necessary to achieve the proper color rendition for an image. Many factors impact the coloration of an image capture, including lamp voltage, the coloration of the glass used in the slide, and the coloration of the glass used in the lenses of the microscope's objectives. To minimize the impact of these factors, use the Compute White Balance feature. When you perform a white balance calculation, SPOT samples the color of the light from your light source after it has passed through the slide and the objective, and then calculates the exposure values needed to produce images where the white areas are white and the color areas are true.

You should perform a new white balance calculation when:

- Starting a new image capture session.
- Changing the lamp voltage.
- Switching to a different objective on the microscope.

When you open an image setup, the Image Setup screen displays the white balance values that were last saved to the Image Setup. If you capture an image without computing a new white balance, SPOT uses these default values.

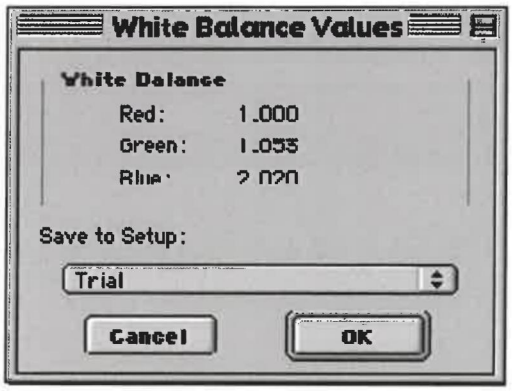
To compute new white balance values for an image setup, follow these steps:

1. Set the illumination and the objective that you will use for the image capture.
2. Show the camera a sample of white light. This step varies according to microscopy technique and sample type, as shown in the table below:

Image Type	White Balance Technique
Brightfield-transmitted light	Position the slide so that the specimen is not in the field of view, but light is going through the slide near the specimen.
Darkfield-transmitted light	Perform the white balance using the brightfield mode on your microscope. See Brightfield – transmitted light above.
Fluorescence	Use the Match Color feature. Refer to Ch. 8 – Viewing, Editing, and Annotating Images for more information.
Nomarksi DIC/Hoffman	**For Nomarski DIC:** Adjust the Nomarski prism until the image is in either the "brightfield" or the "gray scale" view. **For Hoffman:** Perform the white balance directly on the Hoffman image.
Phase contrast	Position the slide so that the specimen is not in the field of view, but light is going through the slide near the specimen.
Polarized light	Pull all polarizers and compensators out of the light path and then position the slide so that the specimen is not in the field of view, but light is going through the slide near the specimen.
Brightfield-reflected light	Replace your sample with a mirror.
Darkfield-reflected light	Perform the white balance using the brightfield mode on your microscope. See Brightfield – reflected light above.
Stereo	Using an EPI-illuminator (shining a light on the top of your specimen from off to one side), replace your sample with a white sheet of paper.

3. From the Camera menu, select the Compute White Balance Values option, or use the toolbar and click on the Compute White Balance Values button. The Compute White Balance window appears.

4. Click on Begin to start the calculation. The message **Computing white balance values** displays, as the camera samples the light. When the camera finishes calculating the white balance values, SPOT displays the computed white balance values.



> *Note: Due to the inherently poor blue light sensitivity of CCD chips and the energy distribution of halogen bulbs, the blue value is often much higher than the red and green values.*

5. To change the setup that the new white balance values are saved to, select a setup from the drop - down box.
6. Click on OK. The program returns to the main editing and viewing window.
7. To edit the white balance values that you saved, open the image setup (i.e., Brightfield from the previous example). The values should appear in the Red, Green, and Blue selection bars. Edit the values as needed.

Exposure Time (User Defined Exposure)

There are three options for entering user-defined exposure times. With the SPOT camera, you can:

- Manually enter the value.
- Allow the camera to calculate the value.
- Recall the exposure time and the gain used for the previous image capture.

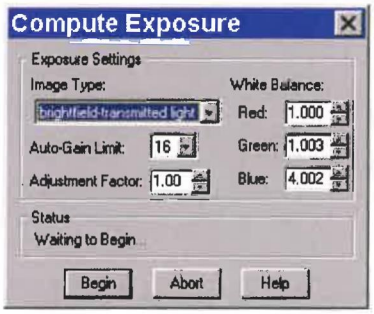
When you select the User-Defined option on the Image Setup, a default value appears for the Exposure Time.

To manually enter new exposure value(s) follow these steps:

1. Ensure that the User-Defined Exposure box is selected.
2. Select either the **msec** (milliseconds) option or the **sec** (seconds) button.
3. Either use the selection arrows or type in a value (in seconds) for the exposure time.
   - The minimum exposure time is **1 millisecond (.001 seconds)**.
   - The maximum exposure time is **536,000 milliseconds (536 seconds)**.
4. Click on OK to save the settings.

To have the camera calculate the exposure time, follow these steps:

1. Close the Image Setup window.
2. Close the Image Setups dialog box.
3. From the Camera menu, select Compute Exposure.
4. The Exposure Settings for the current image setup appear. If you decide to change the settings, you can do so from the Compute Exposure window. Any changes that you make will be saved to the current setup.



5. Click on Begin. The Status message changes to **Computing exposure...** as the camera samples the light. When the camera finishes, the Exposure window displays the calculated exposure time and gain.

> *Note: For information on entering Image Type, Auto-Gain Limit, Adjustment Factor, and White Balance, refer to the appropriate section in this chapter.*

6. Select an image setup from the Save to Setup scroll bar. The current image setup is the default.
7. Click on OK. When you open the Image Setup window, the new exposure value(s) appear.

> *Note: When you save the calculated exposure value to an image setup, it automatically changes the setup from Auto – Exposure to User Defined.*

To recall the exposure time and gain from the previously captured image, follow these steps:

1. From the Camera menu, select the Recall Previous Exposure option. The Exposure window displays the exposure time and gain used for the previously captured image

2. Select the setup that you want to save the exposure settings to, and click on OK. The settings are saved to the image setup that you specify.

Note: When you save the previously used exposure value to an image setup, it automatically changes the setup from Auto – Exposure to User Defined.

Gain (User-Defined Exposure)

When you define your own Exposure, you can enter the precise gain that the camera will use for an image capture. You can enter a specific gain in one of three ways:

- You can manually enter the gain by selecting one of the five gain options from the selection list.
- You can let the camera compute the best gain (and exposure times) by using the Compute Exposure feature (see the previous section).
- You can use the Recall Previous Exposure feature to recall the gain from the previous image capture (see the previous section).

To manually enter a gain, follow these steps:

1. Ensure that the User-Defined Exposure option is checked.
2. Select one of the five gain options from the list. If the pixel bit depth is set to 12 bpp or 36 bpp, the 16 gain value is unavailable.

To use the gain that the camera selects, follow the steps (in the previous section) listed for computing exposure times. The Exposure window, in addition to the optimal exposure times, displays an optimal gain. This gain is automatically added to the image setup that you specify in the Save to Setup box.

Note: The Compute Exposure window displays the Auto-Gain limit defined for the current image setup. This limits the gain options that the camera can choose from. If you do not want to use the Auto-Gain Limit shown, it can be changed from the Compute Exposure window.

External Shutter Lag

When using an external shutter - to shutter a fluorescence illuminator, for example - there is a delay between the application of voltage and the shutter opening to 100 %, as is the case with any mechanical shutter (see the graph at right). This delay between the application of voltage and the full opening of the shutter is referred to as the *external shutter lag*. SPOT Insight cameras feature electronic shutters that open almost instantaneously. When using an external shutter with a SPOT Insight camera, then, the SPOT shutter needs to be delayed until the mechanical shutter is fully open. To avoid triggering the electronic shutter before the external shutter has had a chance to open fully, check the **External Shutter Lag** box, and enter the external shutter lag time value in milliseconds. To determine the correct value, refer to the specifications guide that accompanies the external shutter.

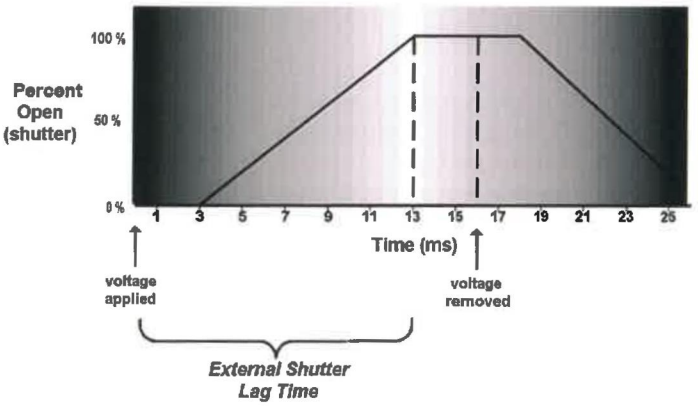


Figure 5- 2: External Shutter Lag Time

Minimum Exposure

Because SPOT Insight cameras are capable of extremely short exposure times, light sources with intensities that vary with the waveform of the 60 Hz line supply (e.g., fluorescent lamps 120 volt incandescent bulbs, and 6 volt 20 watt halogen lamps) can cause the Live Image window to flicker and result in incorrect brightness and color balance readings in captured images. The Minimum Exposure option allows you to correct for these problems by limiting the camera to a minimum exposure time that is greater than the pulse interval of the light source. Consider the illustration to the right. In this case, the pulse interval of the fluorescent light source is 8.3 ms. Entering a Minimum Value of 10 ms ensures that the camera's exposure time is greater than the interval between the pulses of the light source. To enter a minimum exposure, ensure that you have selected the **Auto-Exposure** option, check the Minimum Exposure box, and enter a value in milliseconds.

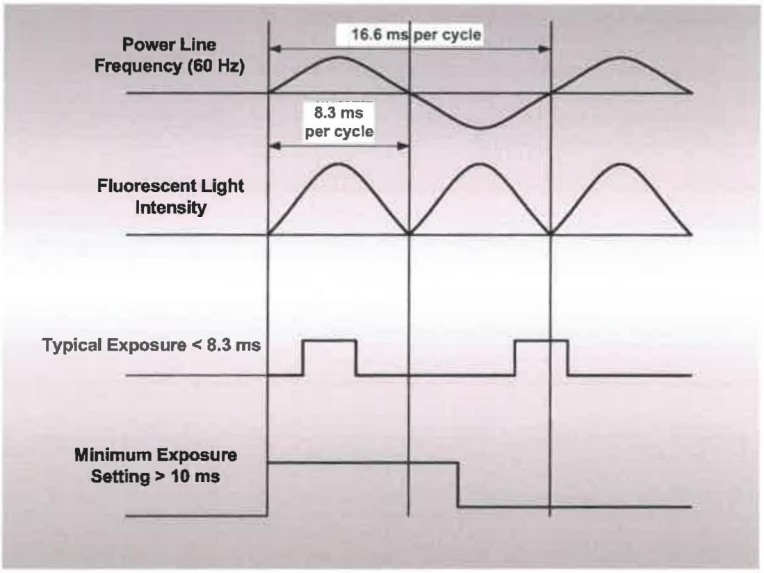


Figure 5- 3: Setting a Minimum Exposure to Accommodate Varied Light Sources (Advanced Mode)

Note: For countries with 50 Mhz power line frequency, a minimum exposure of 15 ms is recommended.

Chip Imaging Area

SPOT allows you to use any rectangular portion of the CCD chip for image capture. Using a portion of the chip is helpful in cases where you want to:

- Keep file sizes small.
- Shorten download times.
- Avoid areas of the image with optical problems (e.g., vignetting).

There are three image area options:

- **Full Chip** – Capture the entire area of the CCD chip.
- **Center** – Capture a rectangular part of the CCD chip's area that has the same height - to - width ratio as the full chip, and is centered on the center of the full chip. The percentage that you enter is the percentage of full chip width that the new area's width will be.

  To capture a centered proportion of an image, follow these steps:

  a) On the Image Setup window, check the Center option.

  b) Enter the percentage of the image that you want to capture. The minimum percentage is 10 and the maximum is 100.

  c) Click on OK to save the image setup.

- **Region** –Capture a specific part of the CCD chip.

  If you have pre-determined the exact pixel values of the area that you want capture, follow these steps:

  a) On the Image Setup window, check Region.

  b) Enter the pixel values for the Left and Right columns and the Top and Bottom rows that form the boundaries of the region that you want to define.

  c) Click on OK. The camera captures the region that you selected.

  If you do not know the exact pixel values of the area that you want to capture, do the following:

  a) Click on the Full Chip option.

  b) From the Camera menu, select the Get Image (exposure from setup) option. The image capture appears on the screen.

  c) Position the cursor at the upper left edge of the image region that you want to define.

  d) Press and hold the left mouse button, and drag the cursor across the screen. SPOT highlights the area that you selected with a dotted border.

  e) From the Camera menu, select the Set Chip Imaging Region option. The Chip Imaging Region window displays the pixel values for the region that you selected.

  f) Either click OK to save the region values to the current image setup, or select a different image setup.

Post-Processing

The final aspect of defining an image setup is to determine the post-processing adjustments (corrections) that you want the SPOT camera/software to make. Each option is described in the sections that follow. Some options are available only for the Insight Color camera or only for the Insight B/W (monochrome) camera - this is indicated parenthetically next to the heading.

Black Level Subtract (12 BPP) (Insight B/W)

The amplifier in the SPOT camera has been adjusted so that the average black (no light) pixel value is between 20 and 25, rather than zero for 12 bit (4,095 full scale) downloads. This offset is called the **black level** (sometimes referred to as the "pedestal"). Before each picture, the camera samples the black level by taking a picture with the shutter closed. The software then downloads the pixel values in the first row of the CCD chip, averages them, and stores this value as the black level. When capturing 8-bit monochrome, 24-bit RGB and 36-bit RGB images, the software automatically subtracts the black level from all pixel values so that black pixels are 0 (black) rather than 20-25 (dark gray), and colors are represented accurately.

When capturing 12-bit monochrome images, you can turn off the automatic Black Level Subtract. This is a useful option in cases where you need high accuracy brightness measurements. For example, you may want to adjust the black level of an image on a pixel-by-pixel basis by subtracting a second picture, taken with no light (a "dark frame"), from the original image. When high accuracy measurements are needed, the automatic Black Level Subtract is a problematic because the Black Level is an *average* of a sampling of black pixel values. Some values are higher, and some values are lower than the average. Subtracting this average value from a black pixel value that is lower than average would normally result in a negative value. But, since there are no negative values, the result is arbitrarily set to zero and the actual value is lost. This diminishes the accuracy of measurements.

There are two situations in the SPOT software where the automatic Black Level Subtract is *always* turned off:

- When you are acquiring background images (the Get Background Image menu option) and 12 bit is selected as the Pixel Bit Depth on the Image Setup window.
- When you are capturing 12 bit mono images and the Background Subtract option is selected on the Image Setup window.

When you define a new 12 bit monochrome image setup, the Black Level Subtract box is, as a default, checked. To turn off the program's automatic Black Level subtraction, deselect the Black Level Subtract box.

Chip Defect Correction

When you capture an image, the SPOT program automatically compensates for CCD chip defects, so that they do not appear on your images. The Chip Defect Correction option allows you to turn off this automatic correction. This can be useful in cases when you are performing quantitative measurements of pixel brightness values and need to see precisely where chip defects are.

The Chip Defect Correction box is, as a default, checked when you define a new image setup. To turn off the program's automatic chip correction, deselect the Chip Defect Correction box.

Color Enhancement (Insight Color)

The Color Enhancement option optimizes image captures by closely matching the final capture to the image as seen through the microscope. To optimize the final image, the SPOT program processes the raw image data to produce a final image that matches the view seen through the microscope.

Note: Color Enhancement adjustments are not reflected in the live image view –they appear only in captured images. Refer to Ch. 6 – Using Live Images for more details.

Noise Filter

The Noise Filter corrects for the effects of electrical or thermal noise. When capturing dim images with lengthy exposure times, the noise filter acts as a "virtual cooler" for the Insight camera, eliminating "hot pixels."

The Noise Filter checks each pixel on the CCD chip as described below:

1. The program compares the value of each red pixel with the values of the eight neighboring red pixels.
2. If none of the eight neighboring red pixels has a value within ±50 % (i.e., the threshold value that the SPOT program uses) of the value for the red pixel being checked, the value for the red pixel being checked is replaced with the average value of the eight neighboring red pixels.
3. The program repeats this procedure for the values of the green and blue pixels being checked.

To use the Noise Filter, follow these steps:

To filter noise, follow these steps:

1. Check the **Noise Filter** box.
2. Enter a value between **10%** and **100%**. The Threshold value is set to a default of 50%. Refer to the procedure described above for an explanation of the Threshold value.
3. When you capture an image, the program automatically adjusts the image using the Noise Filter value that you entered.

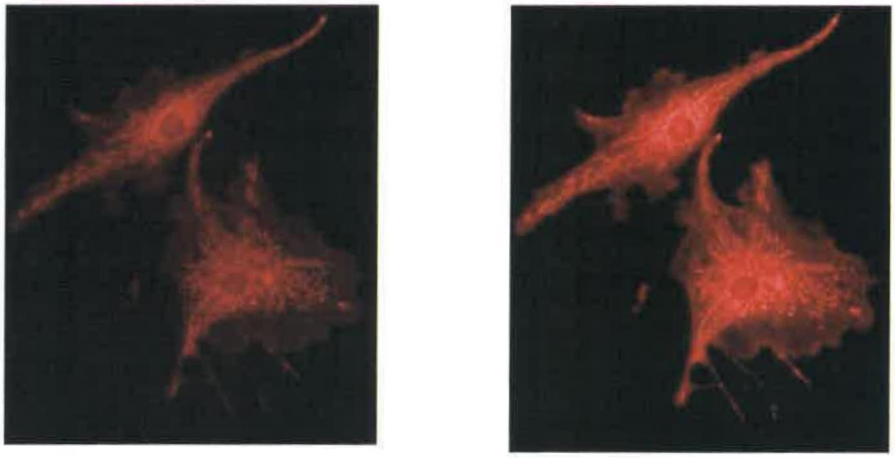
Note: You can also use the Filter Noise *Edit menu option to adjust captured images, as described in Ch. 8 – Viewing, Editing, and Annotating Images.*

Gamma Adjust

Gamma Adjust smoothly lightens darker areas of an image without burning out bright areas or lightening black areas. This is especially useful for capturing/editing images with a wide range of brightness values (dynamic range) such as:

- Fluorescence specimens
- Macroscopic objects with glints, such as printed circuitry

When the bright areas of these types of images are correctly exposed, the darker areas can be so dark that they are in effect invisible. Gamma Adjust can remedy this problem. The figures below illustrate the effect of a gamma adjust on a fluorescent image. The image on the left is the original image, prior to the gamma adjust. The image on the right has been gamma adjusted to a value of 1.75. In this instance, the gamma adjustment results in a better display of detail by lightening the darker areas without burning out bright areas or lightening black areas:



Fluorescence image, no gamma adjustment *Fluorescence image, gamma adjusted to 1.75*

Figure 5- 4: Effects of Gamma Adjustment on a Fluorescence Image

Gamma adjustment corrects an image by creating a new version of the original. To create the new image, the Gamma Adjust function reassigns the RGB values of each pixel in the image according to the curve in the graph at right. This graph demonstrates the basic principles of gamma adjustment:

- Black (pixel value = 0) remains black at all gamma values.
- White (pixel value = full scale) remains white at all gamma values.
- Gamma values greater than one lift the darker areas of the original image into the brighter areas of the new image.
- A gamma curve is smooth - there are no unexpected jumps or cutoffs. This means that when viewing a gamma adjusted image, you will be able to see the details (intensity differences) in both the black and white areas of the image.

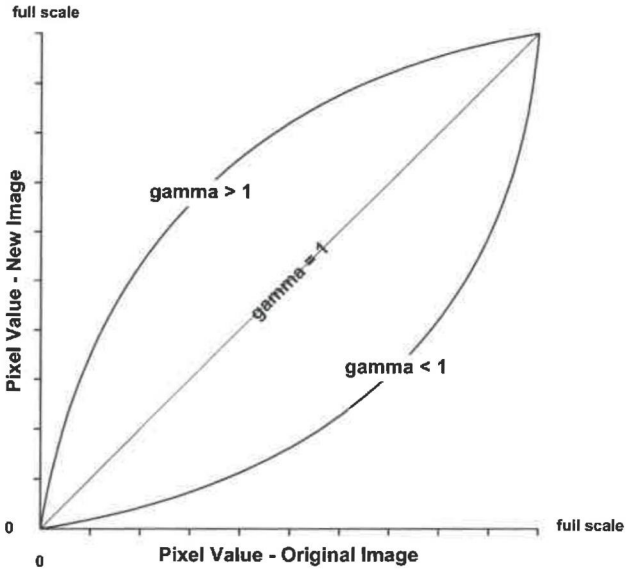


Figure 5- 5: Gamma Curve

On the Image Setup window, you can specify whether or not to automatically apply gamma adjustment to an image capture. This process works well if you know in advance which gamma value works well for a specific class of images. If you are not certain of the best gamma value, you can acquire non-gamma adjusted images, and then use the HSL, HSV, or RGB gamma adjust options on the Edit menu and experiment with the gamma adjust options to find an optimal value. Refer to Chapter 8 - Viewing, Editing, and Annotating Images for details on using the gamma adjust options.

Note: Gamma adjustments in live imaging are controlled exclusively via the Live Image Controls dialog. If you are using the **Snap** *option to capture an image via the Live Image window, the Gamma Adjust setting that you enter on the Image Setup window has no effect on the final image capture .*

To automatically gamma adjust an image after it is captured, follow these steps:

1. Check the Gamma Adjust box on the Image Setup window.
2. Enter a gamma value between .1 and 4.0. Although there is no formula for determining a gamma value, gamma values are typically between 1.3 and 1.7

Note: Although the gamma adjust option is typically used to lighten the mid-tones of an image, entering a value between .1 and 1 will darken the mid-tones.

3. Select one of the following options: **HSL**, **HSV**, or **RGB**.

Insight B/W *If you are using an Insight B/W camera, the color space options are not available. Gamma adjustment is automatically set to use RGB, the recommended color space for monochrome image editing.*

HSL (Hue Saturation Luminance), HSV (Hue Saturation Value), and RGB (Red Green Blue) are three different *color spaces*. A color space is any coordinate system that is used to mathematically represent color.

- The **HSL color space** is an intuitive model based on the three attributes that are common to all colors: hue, saturation, and luminance. The HSL color space assigns a value for each color attribute (e.g., hue, saturation, and luminance). In this color space the brightest saturated color is considered to be 50 % luminance. Above 50 % luminance, saturated colors transition through pastel shades before reaching pure white at 100 % luminance. This process mimics the natural perception of objects illuminated by broadband light sources.
- The **HSV color space** is similar to the HSL color space except that it uses value instead of luminance. In this color space, the brightest saturated color is considered to be 100 % value. This allows you to modify images that contain only saturated colors (e.g., fluorescence) without changing the bright, saturated areas to pastel or white.
- The **RGB color space** assigns a red, green, and blue value to each pixel in an image. This is the method that color computer monitors use to display images.

The process by which the program applies gamma to an image capture differs according to the color space that you select. The following table briefly describes how gamma is applied in each of the three color spaces and lists situations where the use of one color space would be preferable to another.

Color Space	Gamma Adjustment Process	Recommended Usage
HSL	1. RGB pixel values are translated to *HSL* (hue, saturation, and luminance) values. 2. Gamma is applied to the luminance. 3. The HSL values are translated back to the RGB pixels.	The HSL option is ideal for all color images except fluorescence images.
HSV	1. RGB pixel values are translated to *HSV* (hue, saturation, and value) values. 2. Gamma is applied to the value. 3. The HSV values are translated back to the RGB pixels.	The HSV option is ideal for fluorescence images.
RGB	Gamma is applied separately to the red, green, and blue pixel values.	The RGB option is ideal for monochrome images.

4. Click on OK to save the Gamma Adjust settings to the Image Setup. When you capture an image using the setup, the program automatically gamma adjusts the image according to the settings that you defined.

Image Size Double

When you select the Image Size Double feature, the camera *interpolates* between pixels to create an image that is twice as large. The program reads the red, green, and blue brightness values for the original (e.g., 1315 x 1033 pixel) image and interpolates those values to create a new image that has double the number of columns and rows (e.g., 2630 x 2066 pixels). The red, green, and blue values for the additional pixels are calculated based on the RGB values of the original pixels.

The Image Size Double feature helps to reduce the *pixellation* of fine detail that often results when you use lower magnification objectives. When used with low magnification images, the additional pixels created via the Image Size Double feature help to smooth out details; this results in a higher resolution appearance.

Rotate

The Rotate option allows you to define an image capture's on-screen orientation. There are two options:

- **Right** – The image is rotated clockwise 90 degrees.
- **Left** – The image is rotated counterclockwise 90 degrees.

Horizontal Flip

To flip an image display from left to right, check the Horizontal Flip box..

Note: Horizontal Flip is also available as an Edit menu option – Flip Horizontal.

Vertical Flip

To flip an image from top to bottom, check the Vertical Flip box.

Note: Vertical Flip is also available as an Edit menu option – Flip Vertical.

Background Subtract

Background Subtract allows you to subtract a previously acquired Background Image from an image capture. The Background Subtract option is useful when:

- You want to eliminate the irritating background glow seen in some fluorescence images. Sometimes, you will notice this glow while looking at the specimen under the microscope; other times, you will only notice it after you have already captured an image.
- You are doing high accuracy measurements in 12 bit monochrome and want to subtract a "dark frame" from each image to adjust the black level of each pixel. Refer to the Black Level Subtract section of this chapter for more details.

Insight B/W *If 12 bit mono is selected as the Pixel Bit Depth on the Image Setup window, the automatic Black Level Subtract process is always turned off when acquiring background images.*

The Background Subtract option works by taking two pictures of a slide, using the same exposure and gain settings: a foreground image that contains the specimen and the background glow and a background image that contains only the background glow. When you select the Background Subtract option, the program subtracts the background image from the image of the specimen, thereby removing the background glow. The figures below illustrate a fluorescence image before and after background subtraction:

Fluorescence sample prior to background subtraction

Fluorescence sample after background subtraction

Figure 5- 6: Fluorescence Sample Before and After Background Subtraction

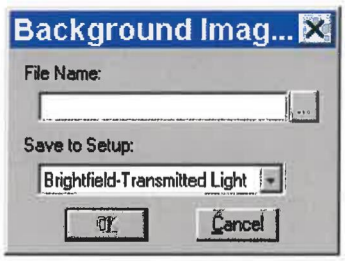
To perform a background subtraction, follow these steps:

1. Ensure that the specimen is under the desired objective and in focus.
2. From the Camera menu, select Get Background Image.
3. If the specimen is not in the objective's field of view, move it into place, and click on Begin. The Get Background Image dialog box displays a status message, as the camera computes the exposure time.

4. When the program has finished computing the exposure time, a dialog box prompts you to "Move to background".

   To capture the background image, move the slide so that only the background portion of the specimen is in the field of view.

5. Click on OK. The Get Background Image window displays the status as the background image is captured.
6. When the program finishes capturing the background image (i.e., the image that will be subtracted from the final image of the specimen), it prompts you for a file name. Enter a name in the File Name box. Because background image files are different for each objective, you might want to name the background file according to the objective's magnification (e.g., 10x).



7. Choose the file save location. If you do not specify a location, The program automatically saves the background image file to the **c:\SpotCam\bkgdimgs** directory (Windows) or **System Folder>Preferences>SPOT 3.4 Prefs>Bkgdimgs** (Mac). To specify a different directory, click on the square to the right of the File Name box and browse for a location.

Note: The default file location varies, depending on where you installed the SPOT program.

8. Specify the image setup that will be associated with this background image file when using Background Subtract. The current setup is the default. When you open the setup, the Background Subtract option is selected.
9. Click on OK.

10. Open the image setup that you specified when you saved the background image (e.g., Brightfield-Transmitted Light).
    - All of the Exposure and Chip Imaging Area options are disabled (grayed).
    - A checkmark appears in the Background Subtract box.
    - The directory path and background file name that you defined in steps eight and nine appear in the Background Subtract dialog box.
11. Click on OK.
12. Move the specimen back into the field of view, and perform the image capture. Refer to Ch. 7 – Taking a Picture, for more details on the image capture process.

Flatfield Correct

For all other SPOT camera lines, the Flatfield Correct option is used to correct for display problems associated with uneven intensity or coloration in illumination, or to correct for artifacts (e.g., dust) in the optical system. Flatfield can still be used for this purpose with SPOT Insight cameras. But, because SPOT Insight cameras use a color mosaic chip, Flatfield correction serves a new primary purpose. In the production process for color mosaic CCD chips, the RGB colors are applied directly to the pixels on the chip. This process often results in an uneven color density around the outer 30-40 rows of the CCD chip, causing a "halo" appearance in some image captures, particularly those with low contrast. The Flatfield option can be used to correct this problem.

Note: Flatfield correction with the SPOT camera refers only to the correction of uneven lighting. It should not be confused with the correction of optical field flatness.

Flatfield correction works by taking a picture of the brightfield background without the specimen in the field of view. This image lets the SPOT software know, on a pixel-by pixel basis, which areas in the field of view are receiving less illumination than the brightest areas. When taking a picture that contains a specimen, the software uses this information to accurately increase the brightness of each pixel in the dimly illuminated areas. The resulting image gives the appearance of an even illumination.

Flatfield Correct is similar to Background Subtract in that it is a process that involves two images. In the case of flatfield correction, the flatfield image and the final image are the two images.

To use Flatfield Correct, follow these steps:

1. Ensure that the specimen is under the desired objective and in focus.
2. From the Camera menu, select Get Flatfield Image. The Get Flatfield dialog box appears.

3. Remove the slide from the field of view.

4. Click on Begin. When the program finishes computing the exposure times and acquiring the flatfield file, the program prompts you to name and save the file:

   a) Enter a name in the File Name box. Because flatfield image files are different for each objective, you might want to name the file according to the objective's m agnification (e.g., 10x).

   b) Specify the image setup that will be associated with this flatfield image file when using the Flatfield Correct option. The current setup is the default. When you open the setup, the Flatfield Correct option is selected.

5. Click on OK. If you do not specify a location, the program automatically saves the flatfield file to the **c:\SpotCam\Fltflds** directory (Windows) or **System Folder>Preferences>SPOT 3.4 Prefs>Flatfields** (Mac).

Note: The default file location varies, depending on where you installed the SPOT program.

6. Move the specimen back into the field of view, and perform the image capture. Refer to Ch. 7 – Taking a Picture, for more details on the image capture process.

Locked

The Locked option allows you freeze the settings for an Image Setup that you have defined. This is useful in cases where multiple users have access to the program and you want to preserve the settings that you defined.

To lock an Image Setup, click on the Locked box. When another user opens the Image Setup, the settings are unavailable for entry until the Locked box is unchecked. The Locked box and the appearance of the window serves as a message that the settings should not be modified.

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Ch. 6 – Using Live Images

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QUICK REFERENCE:

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Using Live Images

The SPOT software allows you to view real-time black and white (Insight B&W) or color (Insight Color) previews of your specimens – i.e., your potential image captures. Using the Live Image preview, you can scan, zoom, focus, and frame images so that they are optimally adjusted for the image capture. After focusing and positioning the image, you can then capture it using one of three options:

- **Get Image (exposure from Setup)** - Capture the image using the settings from the current Image Setup and ignore the Live Image Controls values. This allows you to use one set of values for framing and focusing an image and a different set of values for capturing the image.
- **Snap** - Capture the image using the **area** and **exposure** values from the Live Image Controls dialog (see below). The **Snap** feature is handy because it enables you to adjust the live image until you see what you want, and then use those settings to capture the image – "what you see is what you get." Also because the **Snap** option uses Live Image exposure values, it allows you to avoid the exposure computation that typically precedes image captures.

  Depending on how **Live Image** Preferences (see Ch. 4) are defined, the **Snap** option either uses or disregards the Post Processing options from the current Image Setup (see Ch. 5).

  – *If the **Snapped Images** box is selected,* the Snap option uses all of the Post Processing options from the current Image Setup, except for Background Subtraction and Flatfield Correction.

  – *If the **Snapped Images** box is unselected,* the Snap option ignores the Post Processing options for the current Image Setup, with one exception: if the **Chip Defect Correction** option is selected for the current image setup, the corrections will appear in the image capture, regardless of the Snapped Images preference.
- **Transfer** - You can **Pause** the live image view and **Transfer** (see below) the preview directly to the SPOT editing window. This option is helpful in situations with rapidly changing samples. If you see something that you like, you can pause the live image preview before it changes. Then, you can

transfer the paused image to a SPOT editing window and save it to file. Images saved in this way have two restrictions. First, all transferred images are 8 bit per channel (color). Second, because live images are read out via two amplifiers, the right and left sides of the image might not be perfectly matched.

Depending on how **Live Image** Preferences are defined, the **Transfer** option either uses or disregards the Post Processing options from the current Image Setup (see Ch. 5).

- *If the **Transferred Images** box is selected,* the Transfer option applies the Noise Filter, Color Enhancement, and Image Size Double options as specified for the current Image Setup.
- *If the **Transferred Images** box is unselected,* the **Transfer** option ignores the Post Processing options for the current Image Setup, with one exception: if the **Chip Defect Correction** option is selected for the current image setup (SPOT and RT cameras only) , the corrections will appear in the image capture, regardless of the Transferred Images preference.

Note also the following:

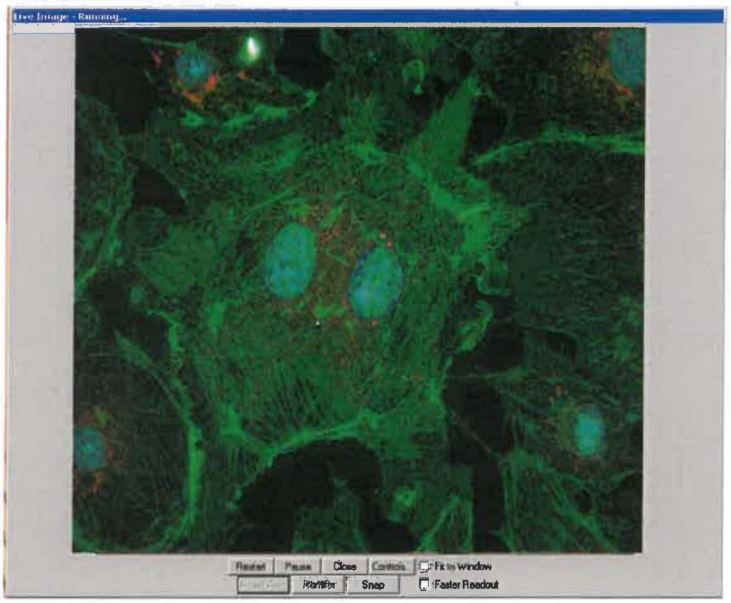
- Image orientation adjustments (i.e., flipping and rotating) specified on the Image Setup are always reflected in the live image view and Snap capture.
- If you crop or zoom in on an image, Snaps and Transfers capture only the cropped or zoomed portion.

Note: With some video cards, you might experience system crashes or display problems in live image mode. To correct these problems, lower the Graphics Acceleration setting in Windows, as described in the Video Card Related Problems *section of the* Installation Guidelines *chapter.*

Note: Occasionally, when viewing a dim live image, you might notice that the right half of the image is brighter or dimmer than the left half. To display images in real time, the Insight camera uses two separate amplifiers to simultaneously read the right and left halves of the CCD chip. With some images, particularly dimmer samples, the brightness of the two halves may not match. This results in a "split" appearance. To avoid this problem with captured images, the Insight camera reads the whole CCD through a single amplifier. A "split" Live Image view is **not** *indicative of what your image capture will look like. Refer to Appendix A: Electronic Imaging Theory and SPOT Cameras for details.*

The Live Image View

To open the Live Image window, either click on the Live Image toolbar button, or select the Live Image option from the Camera menu.



- The **title bar** at the top of the Live Image window displays the status of the live image view - **Running**, **Computing Exposure**, or **Paused**. Double-click on the title bar to "roll up" the live image display. This pauses the live image display. Double-click again to display the whole window and resume the live image display.
- When the **Fit to Window** option is checked, the live image window **cursor** becomes a **zoom tool**. To zoom in on a portion of a live image, click and drag over the area that you want to zoom in on. To return to the non-zoomed image, click on the **Reset Area** button on the bottom of the window.
- When the **Fit to Window** option is not checked, the cursor is a cropping tool. If you select a portion of an image, the program displays only the selected area and discards the rest of the image. To return to the non-cropped image, click on the **Reset Area** button on the bottom of the window.

Note: Due to a flaw in the Windows operating system, monitors that are set to higher resolutions cannot zoom to very high magnification levels. If zoomed in to a high magnification, the monitor displays white instead of the zoomed image.

- The **Full Screen** option (accessed via the View menu or [F 7]) can be used with live images. This option allows you to use the entire screen to view a live image, without the SPOT menus/toolbar and the Windows navigation bar.
  - To access live image viewing options in the full screen view, right click on the live image.
  - To exit the full screen view, either press [F 7] or move the cursor to the top of the screen and click on the **Exit Full Screen Mode** button that appears.
- There are nine options at the bottom of the Live Image window.

Restart	Resample the brightness of the image, compute new exposure times, and restart the live image display. This is useful for updating exposure times when specimen brightness has changed.
Pause/Resume	Freeze the live image window. This is useful when you are changing a specimens and want to preserve the last view. In addition, to use the Transfer option (see below), you must first Pause the live image view. Click on Resume to restart the live image view
Close	Close the Live Image window.
Controls	Open the Live Image Controls dialog box (see below). From this dialog box, you can define the viewing/capture area, and adjust gamma, brightness level, and dim image quality. You can also elect to display either the full chip or a region of interest. Refer to the steps below for more details on the Live Image Controls window.

Fit to Window	Size images to automatically fit the resizable display window.
Reset Area	Return to the non-zoomed view of the live image.
Transfer	Transfer the live image directly to the SPOT viewing window, where it can be edited and/or saved to file. The Transfer button is inactive until you **Pause** the real-time view. The Transfer option is limited in that all images are 8 bits per channel (i.e., because they are from live mode). The Transfer option should not be used for image captures that require highly accurate brightness measurements.
Snap	Capture the live image using the settings from the Live Image Controls dialog. When you click on the Snap button, the **Get Image** status dialogs appear, just as they do when you select **Get Image (exposure from setup)** from the Camera menu. When you use the Snap option to capture an image, you avoid the exposure computation sequence that typically precedes image captures.
Faster Readout	Faster Readout enables you to "speed up" the live image display (higher frame rates) in cases where you are willing to sacrifice some resolution for a higher frame rate.

Live Images are Different Than Captured Images

When you view a live image, keep in mind that the captured version of the image will not be identical to the live image view. This is due to two factors:

- Live images are raw pixel data. Captured images are digitally processed to give a more accurate color rendition. For individual image captures, there is enough time to process the data. For each image in the live image stream, there is not enough time to process the data.
- Live Image gamma adjustments are made in the RGB color space. Captured image gamma adjustments are made in the HSL color space (HSV for fluorescence). This is because gamma adjustments can be made quickly in the RGB color space, but are very slow in the HSV and HSL color spaces. Refer to Ch. 5 – Preparing to Take a Picture for more information on gamma adjustment and color spaces.

Note: HSL and HSV gamma adjustment for captured images applies only to images captured using the Get Image (exposure from Setup) *option. Gamma adjustment for images captured using the* Snap *live image option is always made in the RGB color space.*

Live Image Controls

To adjust the live image view settings, click on the **Controls** button at the bottom of the Live Image window. The Live Image Controls dialog appears.

Binning (Insight B/W)

Binning, as described in Ch. 5 - Preparing to Take a Picture, combines adjacent pixels on the CCD chip, thereby reducing the number of pixels used in the image capture. Binning is useful when you have dim images and want to increase sensitivity and reduce download time. This gives you higher frame rates and less noisy images. But, as binning level increases, image resolution decreases. All of these factors should be considered when you select a binning option.

Area

The Live Image Controls window allows you to view any rectangular portion of the CCD chip.

- **Full Chip** - View the entire chip.
- **Selection** - View a selection that you made with the cursor.
- **Region (from Setup)** - View the region (if any) that is defined for the current image setup.

Use the **Reset Area** button at the bottom of the Live Image window to return to the full chip view.

When you are viewing a live image **Selection**, how this selection is displayed, depends on whether the **Open Images in Fit to Window Mode** and **Always Show Full Chip in Live Image** preferences (see the **Preferences** section in Ch. 4) are checked. The following table illustrates the possibilities:

Fit to Window Checked?	Always Show Full Chip in Live Image Checked?	Selection Display	Live or Captured Image
YES	YES	When you select a portion of the chip, the selection is outlined with a **yellow rectangle.**	LIVE
YES	NO	When you select a portion of the chip the cursor is a **zoom tool.** To zoom in on a portion of a live or captured image, click and drag over the area that you want to zoom in on. To return to the non-zoomed image, click on the **Full Chip** option.	BOTH
NO	YES	When you select a portion of the chip, the selection is outlined with a yellow rectangle. However, because **Fit to Window** is not checked, you will not see the entire chip in the window.	LIVE
NO	NO	When you select a portion of the image, the cursor is a cropping tool. If you select a portion of an image, the program displays only the selected area and discards the rest of the image. To return to the non-cropped image, click on the **Full Chip** option.	BOTH

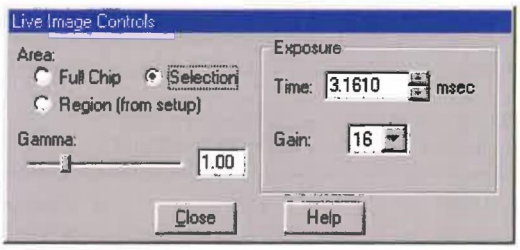
Gamma

Enter a **Gamma** value between **.1** and **4**.
Gamma adjustment smoothly lightens darker areas of an image without burning out bright areas or lightening black areas. This is especially useful for capturing/editing images with a wide range of brightness values (dynamic range) such as fluorescence specimens or macroscopic objects with glints, (e.g., printed circuitry).

- Values between **1** and **4** lighten the mid-tones of the image.
- Values between **.1** and **1** darken the mid-tones of the image.

Because images update very quickly in Live Image mode, gamma adjustments are only displayed in the RGB color space. Gamma adjustments made in the HSL or HSV color spaces would be too slow for live imaging display. Refer to Ch. 5 – Preparing to Take a Picture for more details on color spaces and gamma adjustment.

Exposure



The Exposure options that you see on the Live Image Controls window vary according to The **Auto/User-Defined Exposure** setting for the current image setup:

- *If the current Image Setup is set to **Auto...***
  The Live Image Controls dialog allows you to enter a **Brightness** value and control the dim image quality with **Quality/Speed** slider.
- *If the current Image Setup is set to **User-Defined...***
  The Live Image Controls dialog allows you to enter a specific exposure **Time** value and a specific **Gain** value.

The sections below discuss each option.

Brightness (Auto)

Enter a **Brightness** value between **.25** and **32.**
A brightness value of 1, the default value, gives you the correct image brightness for most images. The Brightness Adjustment control allows you to overexpose or underexpose (darken or lighten) the image area. In many cases, an image has varying levels of brightness; to see a specific area better you might want to lighten or darken the image. When using the Brightness control, the SPOT camera uses an algorithm that combines gain and exposure time to provide the fastest possible image update rate (shutter speed) in live preview mode:

- When overexposing images to make them brighter (i.e., entering values greater than one), the camera first selects the highest possible gain before lengthening the exposure time. Because gain also brightens an image, less exposure time is needed.
- When underexposing images to make them darker (i.e., entering values less then one) the camera uses the lowest possible exposure time before lowering the gain.

Quality/Speed (Auto)

Use the slider to balance speed and quality when previewing dim images.

- Moving the slider toward the **Quality** side increases image quality and decreases frame rates.
- Moving the slider toward the **Speed** side increases frame rates and decreases image quality.

The Quality/Speed measurement corresponds to the Auto-Gain limit setting (see Ch. 5) on the Image Setup window, which allows you to set an upper limit on the gain options that will be available during the Auto-Exposure process.

- When the slider is at the left-most point, the Auto-Gain Limit is 1, which restricts the camera to a gain of 1, giving you higher quality pictures, but lengthier exposure times.
- When the slider is at the right-most point, the Auto-Gain Limit is 32, which allows the camera to select from the six gain options available in Live Image Mode. This shortens exposure times, but decreases image quality.

Note that the highest gain option, 32, is not an option on the Image Setup window, which features only five gain levels, 1, 2, 4, 8, and 16. While a gain of 32 is usually too grainy for captured images, it can be helpful in speeding the display of dim images in live image mode.

For more details on setting an Auto-Gain Limit, refer to Ch. 5 –Preparing to Take a Picture. For more information on the concept of gain, refer to Appendix A: Electronic Imaging Theory and SPOT Cameras.

Auto-Brightness (Auto)

Available only when you are using **Auto** exposure, the **Auto-Brightness** option enables you to maintain a consistent brightness level for changing specimens.

Exposure Time (User-Defined)

From the Live Image Controls window, you can enter a specific exposure time for the image. When you use a specific exposure time, the camera skips the auto-exposure sequence (Computing Exposure) that takes place when you open the Live Image window. This is very useful in cases where exposure times are lengthy, as with fluorescence samples.

To enter new exposure value(s) on the Live Image Controls window, follow these steps:

1. Ensure that the User-Defined Exposure box for the current image setup is selected.
2. Either use the selection arrows or type in a value in milliseconds for the exposure time.
   - The minimum exposure time is **1 millisecond**.
   - The maximum exposure time is **536,000 milliseconds (536 seconds)**.

Gain (User-Defined)

From the Live Image Controls window, you can enter the precise gain that the camera will use for an image capture. Use the drop-down list to select one of the six gain options: **1**, **2**, **4**, **8**, **16**, or **32**. Note that the highest gain option, 32, is not an option on the Image Setup window, which features only five gain levels, 1, 2, 4, 8, and 16. While a gain of 32 is usually too grainy for captured images, it can be helpful in speeding the display of dim images in live image mode.

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Ch. 7 – Taking a Picture

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Introduction: Three Ways to Take a Picture

There are three ways to take a picture with the SPOT camera:

- **Live Image Pause/Transfer**
  After pausing a live image, pressing the Transfer button saves the image to a window. This is useful for capturing rapidly changing samples. Images captured in this way are essentially "frozen" live images. As such, the right and left sides of the images are read out via separate amplifiers and are limited to 8 bit monochrome/24 bit color.
- **Snap**
  Pressing the Snap button when viewing a live image initiates an image capture where the entire image is read out via a single amplifier. Snap image captures avoid the time dedicated to computing exposure by using the exposure time and gain calculated for the live image.
- **Get Image (Toolbar button)**
  Pressing the Get Image toolbar button initiates an image capture where the entire image is read out via a single amplifier using only the parameters from the current image setup. This allows you to use the live image window purely as an aid to framing and focusing the image. Adjustments made to the live image have no impact on the image capture.

These three types of image captures were designed to give you greater flexibility in working with different types of specimens and in how you use live images.

- For more details on the **Live Image Pause/Transfer** and **Snap** options, refer to **Ch. 6 – Using Live Images**.
- For more details on the **Get Image** option, refer to the **Get Image (exposure from Setup)** section below.

This chapter discusses the Get Image type of capture as used in a variety of different procedures. The chapter is divided into two sections:

- **Basic Image Captures** –Standard and sequential image captures.
- **Image Captures in Other Procedures** – Image captures where one capture is used to prepare for another capture.

Basic Image Captures

The SPOT software offers three primary image capture options:

- Get Image (exposure from setup)
- Get Image (last used exposure)
- Get Sequential Images

Get Image (exposure from setup)

Get Image (exposure from setup) captures an image according to the specifications in the Current image setup. Press **[F 12]** to view the current image setup.

To capture an image using the current image setup, follow these steps:

1. Determine or define the image setup that you want to use, and set it as the Current setting (i.e., select the Current option from the Image Setups dialog box).
2. From the Camera menu, select the Get Image (exposure from setup option), or click on the Get Image (exposure from setup) button on the toolbar:

The Get Image window, which indicates Setup name, capture status, and gain, appears. The Status messages are as follows:

a) **Computing Exposure** – The camera is sampling brightness levels in the image in order to determine proper gain and exposure time.

b) **Getting Image** – The camera is downloading the image to the computer.

c) **Shutter open 0:07** – A countdown clock displays when the exposure will last longer than 2 seconds. This informs the user that the electronic shutter is open and counts down the minutes:seconds remaining until it closes.

d) **Processing** – The camera is preparing to display the image.

The newly captured image appears on screen. Following an image capture, several noticeable changes take place in the appearance of the main SPOT screen:

- The Edit and View menus become available.
- The editing, save, and print related toolbar buttons are available (no longer grayed).
- The Get Image (last used exposure), Set Image Region, and Recall Previous Exposures options on the Camera menu are available (no longer grayed).

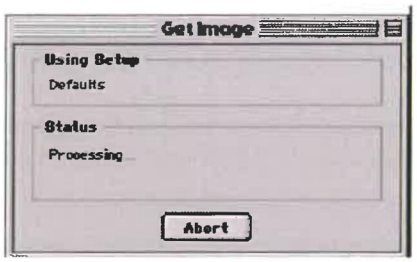
Get Image (last used exposure)

The Get Image (last used exposure) option captures an image using the exposure times and gain from the previous exposure. In all other respects, it works in exactly the same way as the Get Image (exposure from setup) option. This option shortens the time required to capture an image because it skips the initial auto-exposure sequence. This is convenient when you are satisfied with the appearance of an image and want to use the same settings to capture a similar image in the same session. If you close the software, and re-open it, the Get Image (last used exposure) option is unavailable until you capture an image using the Get Image (exposure from setup) option.

To use the Get Image (last used exposure) option, follow these steps:

1. Prepare the microscope and specimen for the next image capture.
2. Either select Get Image (last used exposure) from the Camera menu, or click on the toolbar button.

3. The Get Image status window appears, and the camera captures the image using the last used exposure times. It only displays the exposure time and the gain because the camera no longer needs to sample image brightness.

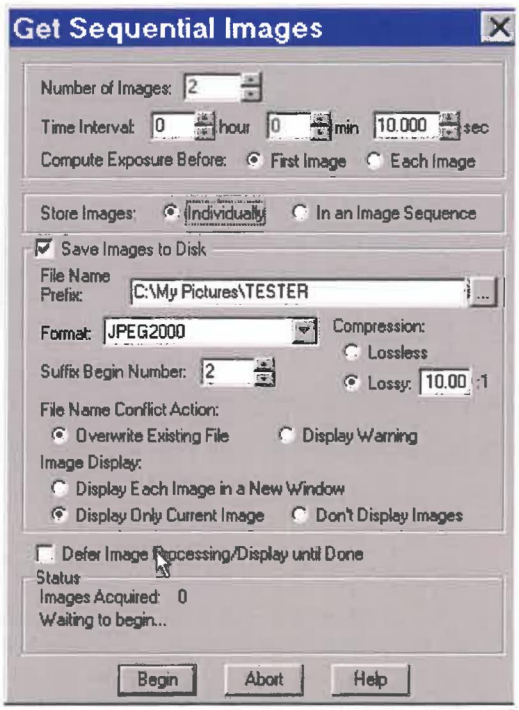


Get Sequential Images

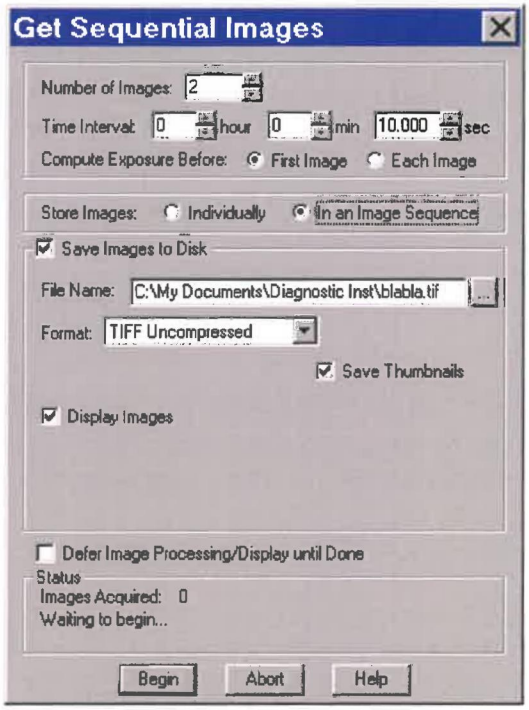
The Get Sequential Images option allows you to capture a sequence of images. SPOT's sequential image capture process enables you to save an image sequence in two different ways:

- You can save a sequence as a group of individual image files.
- You can save a sequence as a single (TIF format), "sequence image file" that is composed of multiple images. Refer to Ch. 8 – Viewing, Editing, and Annotating Images for details on viewing and modifying image sequence files.

The available file name and save options on the Get Sequential Images window vary according to whether you save the sequential images individually or as a sequence, as shown here:


Get Sequential Images
Number of Images: 2
Time Interval: 0 hour 0 min 10.000 sec
Compute Exposure Before: First Image Each Image
Store Images: Individually In an Image Sequence
Save Images to Disk
File Name Prefix: C:\My Pictures\TESTER
Format: JPEG2000
Compression: Lossless Lossy: 10.00 :1
Suffix Begin Number: 2
File Name Conflict Action: Overwrite Existing File Display Warning
Image Display: Display Each Image in a New Window Display Only Current Image Don't Display Images
Defer Image Processing/Display until Done
Status
Images Acquired: 0
Waiting to begin...
Begin Abort Help

Save and name options – individual files


Get Sequential Images
Number of Images: 2
Time Interval: 0 hour 0 min 10.000 sec
Compute Exposure Before: First Image Each Image
Store Images: Individually In an Image Sequence
Save Images to Disk
File Name: C:\My Documents\Diagnostic Inst\blabla.tif
Format: TIFF Uncompressed
Save Thumbnails
Display Images
Defer Image Processing/Display until Done
Status
Images Acquired: 0
Waiting to begin...
Begin Abort Help

Save and name options – image sequence

To use the Get Sequential Images option, follow these steps:

1. Select the image setup that you want to use for the sequential image captures. Either define a new image setup, or select an existing setup from the drop down list on the status bar (bottom right corner).
2. From the Camera menu, select the Get Sequential Images option. The Get Sequential Images window appears. The table on the following page explains each option.

Number/Timing Options

Number of Images Enter the number of images in the sequence of captures. This number can be set up to **2,000**.

Time Interval Enter the time interval between the start of one image capture to the start of the next image capture in hours, minutes, and seconds.

Compute Exposure Before Select either **First Image** or **Each Image**:

- First Image – The program computes the exposure value for the first capture only.
- Each Image - The program computes a new exposure value for each image capture in the sequence.

Storage Options

Store Images Select either **Individually** or **In an Image Sequence**

- Individually – Store each image as a separate file.
- In an Image Sequence – Store all images in a single TIF format file.

The available save and name options depend on your selection here.

Save and Name Options

Save Images to Disk The **Save Images to Disk** box is checked as a default for the Get Sequential Images window.

- If you do not want to save the sequential images/image sequence to file, deselect the box. Deselecting the box makes the remaining save and display options unavailable. You can define the process/display option (**Don't Process or Display Images until Done**), and then click on **Begin** to start the sequence.
- If you want to save the sequential images/image sequence, leave the box selected, and enter the remaining file save and display options, as described below.

The file name of each image (individual images only) in the sequence is made up of two components:

- A **File Name Prefix** that you designate.
- An automatically indexed Suffix Number that starts with the **Suffix Begin Number** that you designate.

File Name Prefix (individual images) File Name (image sequences) This prefix appears before the number of each image capture in the sequence. To enter the File Name Prefix/File Name, you can:

- Type only the name (e.g., **Growth Sequence**) in the box. The program automatically saves the image sequence to the last used directory. If you specify the prefix only, you cannot see exactly where the files are being saved. Thus, to avoid having to search for your files, you should verify the location of the last save.
- Type the full path and file name (e.g., **C:\Spotcam\Growth_Sequence**) in the box to specify the location of the image sequence. When entering the file path and name, you can also specify a new directory simply by entering it in the path (e.g., C:\Spotcam**Flowers**\Growth_Sequence).
- Click on the browse button to select a new file location and file name (prefix). All of the image files are saved with the prefix that you enter. Thus, if your Suffix Begin Number (see below) is one, the file name for the first image in the sequence would be **Growth_Sequence0001**.

Save and Name Options (cont.)

Format Select the file format for the sequence of images.

- For individual files, there are five options: **Windows Bitmap**, **JPEG**, **Mac PICT**, **TIFF Uncompressed** or **TIFF JPEG**.
- For image sequences there are two options: **TIFF Uncompressed** or **TIFF JPEG**.

Suffix Begin Number (individual images) Select the beginning number for the sequence of images. The Begin Number automatically defaults to the first number that follows the previous sequence of images. So, if you captured a sequence of 10 images, 11 would appear as the Begin Number when you next opened the Get Sequential Images window.

The program prefaces numbers with enough zeros to create a four character suffix. For example, if you have 35 images, the first image suffix will be **0001** and the last image suffix will be **0035**. This enables Windows to correctly order images, so that Growth_Sequence0002 appears before Growth_Sequence1000, and not the other way around.

JPEG Quality (1-100) JPEG quality is the level of compression for JPEG images: this option is available only when JPEG or TIFF JPEG (for image sequences) is selected as the image file format. Enter an integer between 1 and 100 to set the JPEG quality for images. A value of one is the most compressed (and lowest image quality), while a value of 100 is the least compressed (and highest image quality).

Compression (JPEG2000) When the JPEG2000 file format is selected, the Get Sequential Images dialog displays compression options:

- **Lossless** – Information is not lost when the image is compressed.
- **Lossy** – Information is lost according to the ratio that you enter: the higher the ratio that you enter, the smaller the file size and the lower the quality of the image.

Save Thumbnails Save thumbnails of each image in the sequence. This option is available only when JPEG or TIFF JPEG is selected as the image file format.

File Name Conflict Action (individual images) Determine how the program will respond if it finds an image file with the same name as an image in the current sequence. Select one of the following options:

- **Overwrite Existing File** – The program overwrites the existing file with the image from the sequence you are capturing.
- **Display Warning** – The program displays a warning message indicating that a file of the same name already exists, and stops the sequential image capture.

Image Display (individual images) Determine how the sequence of images will be displayed. Select one of the following options:

- **Display Each Image in a New Window** – The program opens a window for each image in the sequence. This option works well if you have a short sequence; if you have a long sequence, however, displaying numerous images uses up memory and could interfere with your use of other programs.
- **Display Only Current Image** – The program sequentially displays each image in the same window. Each image displays only until the next image is captured. This is useful for lengthy sequences where you do not want to be concerned with running out of memory.
- **Don't Display Images** – The program automatically saves all of the sequential image files to the specified location without displaying them.

Display Images (image sequences) Select this box to open an image sequence immediately after the capture. To save the image sequence to file without opening it, deselect the box.

Defer Image Processing/Display until Done Wait until the entire sequence is completed before displaying or saving the images.

After you have entered all of the options, click on **Begin** to start the image capture sequence. Refer to Ch. 8 – Viewing, Editing, and Annotating Images for details on viewing and modifying image sequence files.

Image Captures in Other Procedures

In addition to the basic image captures, the Camera menu contains options in which one image capture is used to prepare the camera for the final image capture They include:

- Set Chip Imaging Region
- Compute Exposure
- Recall Previous Exposure
- Compute White Balance Values
- Get Background Image
- Get Flatfield Image

Insight B/W	Insight B/W camera users will not see Compute White Balance Values option (or the corresponding toolbar button).

Because they are used prior to a final image capture, several of these options are discussed as a part of the image setup process. In such cases, the text of the manual refers back to the appropriate section of Chapter 5 - Preparing to Take a Picture.

Set Chip Imaging Region

The Set Chip Imaging Region selects a specific portion of the CCD chip. Using just a small portion of the available area on the CCD chip is useful because:

- The download time will be shorter.
- The file size will be smaller and easier to manage.

To avoid confusion when defining the region of the image that you want to capture, it is important to realize that there are two separate coordinate systems in use:

- The coordinate system of the CCD chip
- The coordinate system of the displayed image

The coordinates displayed on the Status Bar as the cursor is moved over a displayed image are always "displayed image" coordinates. The coordinates displayed in the Set Chip Imaging Region dialog box are always CCD chip coordinates. These coordinate systems match only if the orientation of an image has not changed (i.e., it has not been flipped or rotated).

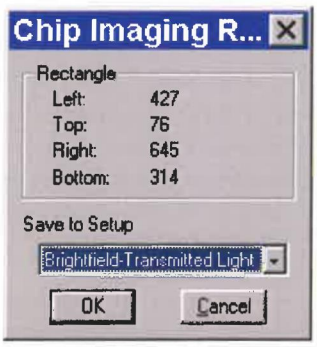
Because the SPOT software tracks orientation changes, you can select a Chip Imaging Region on any displayed image, regardless of whether the orientation has changed: the Set Chip Imaging Region dialog box will correctly display the region of the CCD chip that you designated as the imaging region. However, because of the different coordinate systems, the coordinates that you see on the Status Bar (i.e., when moving the cursor over the displayed image) may not match the coordinates that appear in the Set Chip Imaging Region dialog box if the image was flipped or rotated.

To set an image region, follow these steps:

1. Position the cursor at the upper left edge of the image region that you want to capture.
2. Press and hold the left mouse button and drag the cursor across the screen. A dotted border appears around the region that you select.
3. From the Camera menu, select Set Chip Imaging Region. The Chip Imaging Region window displays the pixel locations for the borders of the region that you selected.

4. Click OK to save the region values to the current image setup, or select a different image setup. The region values are now saved to the designated image setup.

Note: The image setup uses the specified region values for all subsequent image captures. Refer to Ch. 5 – Preparing to Take a Picture for other region capture techniques.



5. Recapture the image using either Get Image (exposure from setup) or the Get Image (last used exposure) option. The new image capture displays only the region that you selected.

Compute Exposure

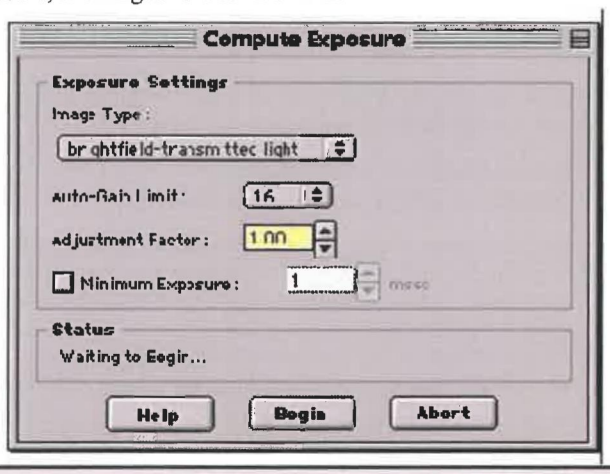
Compute Exposure determines the correct exposure time and gain for an image. By calculating exposure times and gain ahead of time, you can avoid the auto-exposure sequence that takes place each time that you use Get Image (exposure from setup). Using Compute Exposure also changes your current image setup:

- The computed exposure values are added to your current image setup.
- The current image setup, if set to Auto-Exposure, is changed to User-Defined.

To compute exposure, follow these steps:

1. Select Compute Exposure from the Camera menu. The Compute Exposure window displays the image setup options (from the current setup) that directly affect the computation. The entries for these options can be modified from this window. Modifying the options also changes the current image setup.

Insight B/W	Insight B/W camera users will not see white balance values on the Compute Exposure window.



Note: For information on entering Image Type, Auto-Gain Limit, Adjustment Factor, and White Balance, refer to Ch. 5 – Preparing to Take a Picture.

Click on Begin. The Status message changes to **Computing exposure...** When the camera finishes sampling the light, the Exposure window displays the values.

2. Select an image setup from the Save to Setup drop-down box. The current image setup is the default.

Note: Saving an exposure value to an image setup changes the setup from Auto-Exposure to User-Defined Exposure.

3. Click on OK. When you open the Image Setup window, the new exposure value displays.
4. Select Get Image (exposure from setup) to capture the image using the new exposure values.

Recall Previous Exposure

Recall Previous Exposure assigns the exposure time and gain from your last image capture to an image setup. The Recall Previous Exposure feature is available only after you have taken a picture.

To recall previous exposure values, follow these steps:

1. Capture an image.
2. From the Camera menu, select Recall Previous Exposure. The Exposure window displays the value from the last image capture (see above).

Note: If you change the current image setup, the Recall Previous Exposure option will be unavailable until you take another picture.

3. Select an image setup from the drop-down box.
4. Click OK.
5. Use Get Image (exposure from setup) to capture an image with the new exposure and gain values.

Compute White Balance Values (Insight Color)

White balance is the ratio of red, green, and blue exposure times necessary to achieve the proper color rendition for an image. Many factors impact the coloration of an image capture, including the lamp voltage, the coloration of the glass in the slide, and the coloration of the lenses in the microscope's objectives. To minimize the impact of these factors, use the Compute White Balance feature. When you perform a white balance calculation, SPOT samples the color of the light from your light source after it has passed through the slide and the objective, and then calculates the exposure values needed to produce images where the white areas are white and the color areas are true.

You should perform a new white balance calculation when you:

- Start a new image capture session.
- Change the lamp voltage.
- Switch to a different objective on the microscope.

When you open an image setup, the Image Setup screen displays the default white balance values for that Setup, which are the values that were last saved to the Image Setup. If you capture an image without computing a new white balance, SPOT uses these default values. Refer to Ch. 5 – Preparing to Take a Picture for a step by step description of how to compute a white balance.

Get Background Image

Get Background Image is the first step in the background subtract process described in Ch. 5 - Preparing to Take a Picture. Background Subtract allows you to subtract a previously acquired Background Image from an image capture. This is useful when:

- You want to eliminate the irritating background glow seen in some fluorescence images. Sometimes, you will notice this glow while looking at the specimen under the microscope; other times, you will notice it only after you have already captured an image.
- You are doing high accuracy measurements in 12 bit monochrome and want to subtract a "dark frame" from each image to adjust the black level of each pixel. Refer to the Black Level Subtract section in Ch. 5 for more details.

Insight B/W	*If 12 bit mono is selected as the Pixel Bit Depth on the Image Setup window, the automatic Black Level Subtract process is always turned off when acquiring background images.*

The figures below illustrate the effect of a background subtraction on a fluorescence image:

Fluorescence sample prior to background subtraction

Fluorescence sample after background subtraction

Background subtraction works by taking a picture of the background glow only, without the specimen in the field of view, and then automatically subtracting this image from pictures taken with the specimen in the field of view. When you get a background image, you are taking the first of the two pictures used for the background subtraction. This image is the one that the camera subtracts during the final image capture process. For a detailed description of the steps involved in the background subtract process, refer to Ch. 5 - Preparing to Take a Picture.

Insight B/W	*If 12 bit mono is selected as the Pixel Bit Depth on the Image Setup window, the automatic Black Level Subtract process is always turned off when acquiring background images.*

Get Flatfield Image

For all other SPOT camera lines, flatfield correction is used to correct for display problems associated with uneven intensity or coloration in illumination, or to correct for artifacts (e.g., dust) in the optical system. Flatfield can still be used for this purpose with SPOT Insight cameras. But, because SPOT Insight cameras use a color mosaic chip, Flatfield correction serves a new primary purpose. In the production process for color mosaic CCD chips, the RGB colors are applied directly to the pixels on the chip. This process often results in an uneven color density around the outer 30-40 rows of the CCD chip, causing a "halo" appearance in some image captures, particularly those with low contrast. The Flatfield option can be used to correct this problem.

Note: Flatfield correction with the SPOT camera refers only to the correction of uneven lighting. It should not be confused with the correction of optical field flatness.

Get Flatfield Image is the first step in the Flatfield Correct process described in Ch. 5 - Preparing to Take a Picture. Flatfield correction works by taking a picture of the brightfield background without the specimen in the field of view. This image lets the SPOT software know, on a pixel-by pixel basis, which areas in the field of view are receiving less illumination than the brightest areas. When taking a picture that contains a specimen, the software uses this information to accurately increase the brightness of each pixel in the dimly illuminated areas. The resulting image gives the appearance of an even illumination.

For a detailed description of the steps involved in the flatfield correction process, refer to Ch. 5 - Preparing to Take a Picture.

Ch. 8 - Viewing, Editing, and Annotating Images

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Introduction

After capturing an image, you can choose from a variety of viewing, editing, and annotation options that allow you to customize the appearance of the final image. These features are described in the following sections:

- Viewing Captured Images
- Working with Image Sequences
- Editing Captured Images

Note: These menus are available only when an image is on-screen.

Viewing Captured Images

The View menu options include:

- Fit to Window
- Zoom In/Out
- Zoom Level
- Show Grid Lines
- Show Pan Window
- Show Sequence Controls
- Image Date/Time
- Full Screen

Note: The Show Grid Lines option is available only after you have zoomed in (8x or 16x) on an image.

Fit to Window

Fit to Window allows you to view the whole image, regardless of the window size. Typically, reducing the size of a window also cuts off part of the image. The Fit to Window option solves this problem by resizing the image so that it is always the same size as the window.

Note: The View menu options supplement the standard Windows viewing options (e.g., Cascade, Tile Horizontal, etc...) that are found on the Window menu. Refer to the Windows on-line help for more information on these options.

To use Fit to Window, select (i.e., click on) the image that you want to modify and, from the View window, select Fit to Window.

Zoom In/Out

1. Either select Zoom In/Out From the View menu, or click on the Zoom toolbar button:
2. The cursor changes to a magnifying glass, and the Zoom toolbar button becomes active. The precise reference point for the zoom location is indicated by the white dot in the center of the magnifying glass. The row and column numbers at the bottom of the screen indicate the exact position of the white dot as you move the magnifying glass over the image.

3. Use the mouse to zoom in or zoom out:
   a) Position the mouse over the center of the area that you want to zoom in on.
   b) Either left click to zoom in 2x, or right click to zoom out 2x.
   c) To return to the normal view mode, de-select the Zoom option.

The Zoom feature works in conjunction with the pan window (see **Show Pan Window** below), which allows you to see where you are in relation to the larger image while zoomed in.

Note: All of the standard save, view, and edit options, with three exceptions – **Add Calibration Mark**, **Add Measurement**, *and* **Annotate** *– can be used while the program is in zoom mode. If you need to annotate a zoomed image, use the* **Zoom Level** *option (see below).*

Note: With some video cards, you might experience display problems when zooming in on captured images. To correct these problems, lower the Graphics Acceleration setting in Windows, as described in the Video Card Related Problems *section of the* Installation Guidelines *chapter.*

Zoom Level

The **Zoom Level** option, like the **Zoom In/Out** option enables you to view zoomed images. However, unlike the Zoom In/Out option, you have the option to add annotations, including text, calibration marks, and measurements, to the zoomed image. To use the Zoom Level option , select **Zoom Level** from the View menu and select a percentage option from the list.

Like the Zoom In/Out option, the The Zoom Level option can be used in conjunction with the pan window (see **Show Pan Window** below), which allows you to see where you are in relation to the larger image while zoomed in.

Note: With some video cards, you might experience display problems when zooming in on captured images. To correct these problems, lower the Graphics Acceleration setting in Windows, as described in the Video Card Related Problems *section of the* Installation Guidelines *chapter.*

Show Grid Lines

Show Grid Lines clearly defines pixel borders, which are often invisible in low contrast areas of an image. Clearly defined borders help in positioning the cursor at an exact row and column location (i.e., using the readouts at the bottom of the screen), which then allows you to view the brightness value for the individual pixel.

Note: When using the **Zoom In/Out** *option, all of the standard save, view, and edit options, with three exceptions –* **Add Calibration Mark**, **Add Measurement**, *and* **Annotate** *– can be used while the program is in zoom mode. If you want to annotate a zoomed image, use the* **Zoom Level** *option (see above).*

To show the grid lines on an image, follow these steps:

1. Position the magnifying glass in the center of the area that you want to zoom in on.
2. Zoom to either 8x or 16x. If you are using the **Zoom Level** option, select a percentage of 800 or greater.
3. From the View menu, select the Show Grid Lines option. Grid lines appear around each pixel.

Show Pan Window

Show Pan Window enables you to navigate around an image while zoomed in at high magnifications. When you select the Show Pan Window option, the program highlights your location in relation to the whole image, and, by dragging the highlighted area around the pan window, you can explore the image, piece by piece. Without the Show Pan Window option, the process of moving to a new point while in high magnification would involve several steps:

1. Zooming in to the first area of interest.
2. Zooming out to determine where you are.
3. Determining a new area of interest.
4. Re-zooming in to the new area of interest.

This tedious process is replaced by the pan window. When you select Show Pan Window, the whole image appears in the smaller Pan Window. The zoomed-in area of the image is represented by a "color negative" area over the smaller version of the image in the Pan Window.

To use the pan window, follow these steps:

1. From the View menu, select Show Pan Window:

Note: The pan window can be opened at any level of magnification.

The pan window appears, superimposed upon the image. The illustration to the right shows the pan window as it is used when zoomed in on an image.

2. Position the cursor on the image in the pan window: it changes from a magnifying glass to a four arrow pointer.
3. To move to a different area of the image, either left click on the rectangle and drag it to the new location, or left click on the new location.

Show Sequence Controls

The Show Sequence Controls option displays the play controls for image sequences. This option appears only when an image sequence is open. Using the Image Sequence dialog you can:

- Play all or part of an image sequence in forward or reverse order.
- Set the display interval between images.
- Set the action that takes when the image sequence finishes playing – i.e., stop, restart, or reverse.

Refer to the **Working With Image Sequences** section for details.

Image Date/Time

The Image Date/Time feature pulls up the date and time associated with the capture of an image. When you capture an image, the SPOT software remembers the date and time of the image capture. When you save the image, even if this is many hours later, the program sets the **Created** date and time of the image file to the date and time of the original image capture.

When image files are transferred over networks, the **Created** date and time is sometimes reset to the date and time of the transfer, making it an unreliable indicator of the image capture time. Because TIFF and JPEG format images have tags both for the Created date and the original capture date and time, they are not affected by network transfers. If reliable image capture date and time information is important to your work, you should save images in either TIFF or JPEG format.

When you select the Image Date/Time option from the View menu, a dialog box displays the date and time associated with the capture.

- For non-TIFF, non-JPEG format image files, this is the **Created** date/time, as explained above.
- For TIFF and JPEG format image files, this is the TIFF/JPEG tag date and time.
- For SPOT Database images this is the original date and time that was stored in the database file.

Full Screen

View a full screen version of an image – i.e., without the SPOT menus or toolbars or the Windows navigation bar. This option allows you to utilize the entire window for previewing images. Press **[F 7]** to view a full screen image. Press **[F 7]** again to exit the full screen view. Alternatively, you can exit the full screen view by moving the cursor to the top of the window and clicking on the **Exit Full Screen Mode** button that appears. While working in Full Screen mode, Windows users can access SPOT menu options using standard keystroke combinations, as illustrated in the table below:

[Alt] + A =	Attributes (Report mode)	**[Alt] + H** =	Help
[Alt] + C =	Camera	**[Alt] + O** =	Object (Report mode)
[Alt] + D =	Database (Advanced mode)	**[Alt] + Q** =	Sequence
[Alt] + E =	Edit	**[Alt] + S** =	Setup
[Alt] + F =	File	**[Alt] + V** =	View
		[Alt] + W =	Window

Working with Image Sequences

There are three places from which you can edit and play image sequence files:

- The **Sequence** menu – Rearrange the order of a sequence, add and/or remove images to and from the sequence, extract images from the sequence, and play the sequence.
- The **Sequence Controls (Image Sequence)** dialog – Set view options and play the sequence.
- The **Edit** menu - Modify/enhance image sequences using the options on SPOT's Edit menu. Refer to **Editing Images in a Sequence** (below) for more information.

Refer to the sections below for details.

Sequence Menu Options

Image sequences are editable. The **Sequence** menu, which is available only when an image sequence is open, displays the available options. They are as follows:

Extract Images

Extract an image or images from a sequence file. Extracting creates new image(s), leaving the original sequence intact. Click on **Extract Images** and enter the number(s) of the image(s) that you want to extract. To extract multiple images from a sequence, separate numbers with commas and/or dashes - i.e., **5, 6, 7** or **5-7** or **1 – 5, 6, 9**.

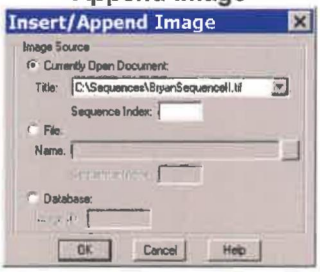
Insert Image

Add an image to the sequence. Using **Insert Image**, you can add an image to a sequence from one of three sources:

- **Currently Open Document** – Add an image from the currently open sequence file. Make sure to specify the **Sequence Index –** i.e., the number of the image in the sequence.
- **File** – Add an image from disk. If the image comes from a sequence file, make sure to specify the Sequence Index.
- **Database (Windows)** – Add an image from a SPOT database. To determine the Image ID, open the database and use the **Search** option. The search results table displays the Image ID for each record.

When you click on **OK**, a dialog prompts you to specify the point in the sequence where you want to insert the image.

Append Image



Add an image to the end of the sequence. Using **Append Image**, you can add an image to a sequence from one of three sources:

- **Currently Open Document** – Add an image from the currently open sequence file. Make sure to specify the **Sequence Index** – i.e., the number of the image in the sequence.
- **File** – Add an image from disk. If the image comes from a sequence file, make sure to specify the Sequence Index.
- **Database (Windows)** – Add an image from a SPOT database. To determine the Image ID, open the database and use the **Search** option. The search results table displays the Image ID for each record.

Remove Images

Remove an image or images from a sequence file. Removing an image deletes the image from the sequence, which is automatically reordered. Click on **Remove Images** and enter the number of the image(s) that you want to extract. To extract multiple images from a sequence, separate numbers with commas and/or dashes - i.e., **5, 6, 7** or **5-7** or **1 – 5, 6, 9**.

Move Image

Move an image or image(s) to a different point in the sequence. Click on **Move Image** and enter the number(s) of the image(s) that you want to move. To move multiple images from a sequence, separate numbers with commas and/or dashes - i.e., **5, 6, 7** or **5-7** or **1 – 5, 6, 9**.

Play Forward	Continuously play the image sequence forward.
Play Reverse	Continuously play the image sequence backward.
Stop Play/Resume Play	Stop play if the sequence is running; resume play when it is stopped.
Step Forward	Move forward one image.
Step Reverse	Move backward one image.

Image Sequence Controls

The **Sequence Controls (Image Sequence)** dialog enables you to play image sequences and to set the play options.

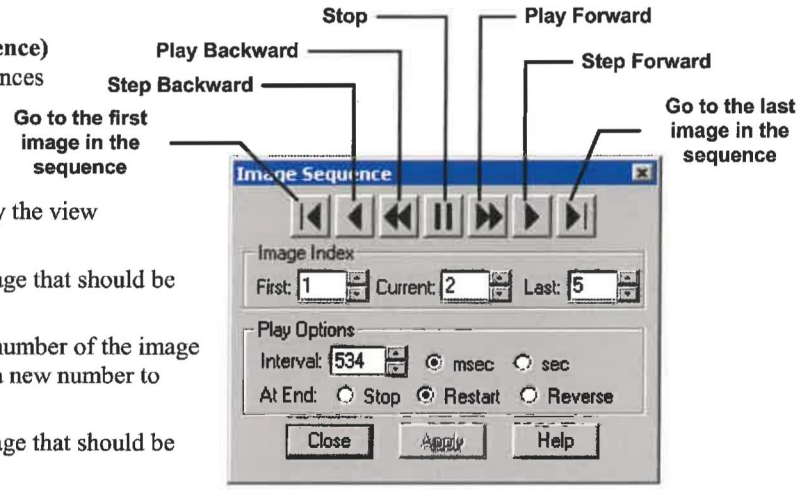


Image Index

Use the **Image Index** options to specify the view parameters of the sequence.

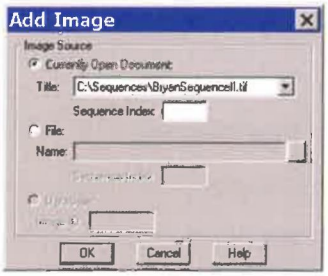
- **First** - Enter the number of the image that should be first in the sequence.
- **Current** – This field displays the number of the image you are currently viewing. Enter a new number to view a different image.
- **Last** – Enter the number of the image that should be last in the sequence

Play Options

Use the **Play Options** to control how the image sequence displays when playing.

- **Interval** – The time interval between each image when the sequence is playing. Enter a number in milliseconds or seconds.
- **At End** – The action that takes place when the sequence finishes playing.
  - **Stop:** The sequence stops at the last image in the sequence.
  - **Restart:** The sequence begins playing from the beginning.
  - **Reverse:** The sequence reverses play when it reaches the end.

Editing Images in a Sequence



Images from a sequential image file can be edited using the **Edit** menu options. This allows you to specify the individual image or images (including the entire sequence) to which the edits are applied.

- In some cases – e.g., **Combine Images** – the dialog prompts you to specify the **Sequence Index** – i.e., the number of the image within the sequence.

- In other cases, as with the **Adjust RGB** options, SPOT prompts you to specify whether the edit applies only to the current image in the sequence, all of the images in the sequence, or a specific subset of images in the sequence. For example, when editing an image sequence, if you select **Edit >Adjust RGB > Adjust Brightness (additive)**, the **Additive Brightness Adjust** dialog appears. After you make your edits and click on **OK**, the **Edit Images** window prompts you to specify the image or images that the edit will apply to, as shown at right.
  - **No Other Images** – The edit applies only to the current image – i.e., the on-screen image.
  - **All Other Images in Sequence** – The edit applies to all of the images in the sequence.
  - **Specified Images** – The edit applies to the images that you specify. Enter the number(s) of the image(s) in the box at the bottom of the dialog. To specify multiple images, separate numbers with commas and/or dashes - e.g., **5, 6, 7**; or **5-7**; or **1 – 5, 6, 9**.

Edit

The Edit menu contains six categories of editing options, as illustrated in the following table:

Editing Category	Menu Option
Basic Editing Functions	Undo Copy to Clipboard
Image Sizing and Position	Resize Rotate Right Rotate Left Flip Horizontal Flip Vertical Crop
Image Quality	Adjust RGB Adjust HSL Adjust HSV Filter Make Negative Convert to Gray Scale Set Palette (monochrome) Match Color (RGB)
Customization	Change Pixel Bit Depth Subtract Image Merge Images Duplicate Image

(cont.)

Editing Category	Menu Option
Annotation Calibration and Measurement Marks Custom Image Annotation	Add Calibration Mark Add Measurement Annotate
Image Memos	Memo

Basic Editing Functions

The Edit menu contains several functions that are common to most operating systems.

Undo Undo the last edit that you made. The Undo option only allows for one level of edits: in other words, you can only undo the immediately preceding edit. To Undo an action, select Undo from the Edit menu.

Copy to Clipboard (PC) Copy (Mac) Copy all or part of an image onto the clipboard for insertion into another program. These options are identical to the Windows **[Ctrl C]** command and the Mac **[⌘ C]** command.

1. Press and hold the left mouse button (PC)/mouse button (Mac).
2. Drag the cursor over the image until the dotted rectangle covers the area of the image that you want to copy. If you copy without "cutting out" a specific portion of the image, SPOT selects the entire image.
3. Either select the Copy to Clipboard/Copy option from the Edit menu, or press **[Ctrl C]** (PC)/**[⌘ C]** (Mac). The image is now ready to be pasted into another application.
4. Open the application that you want to paste the image into, and select from the available pasting options.

Cut (Mac) Cut text fields within the SPOT program.

Paste (Mac) Paste text fields in the SPOT program. The Paste option is unavailable (grayed out) for image editing.

Clear (Mac) Clear a text field in the SPOT program. The Clear option is unavailable (grayed out) for image editing.

Select All (Mac) Select text for copying. The Select All option is unavailable (grayed out) for image editing.

Image Sizing and Position

From the Edit menu, you can:

- Re-size images
- Re-position images
- Match the on-screen orientation of a specimen to the microscopic view
- Crop an image to better reflect the area of interest

Resize

Use the Resize option to reduce or enlarge an image. When you reduce or enlarge an image, the resolution and file size change accordingly. The SPOT program maintains aspect ratio so enlarged and reduced images will not be distorted. When you reduce or enlarge an image, keep the following in mind:

- Reduction - Resolution (number of pixels) and file size decrease.
- Enlargement - Resolution (number of pixels) and file size increase.

The trade off for higher resolution images is increased file size. The Resize option lets you specify the percentage by which you want to increase or decrease the linear dimensions of the image. The program reads the red, green, and blue brightness values for the pixels in the original image and uses those values to create a new image where the number of rows and columns is reduced or enlarged by the percentage that you specify.

Two circumstances where you might want to enlarge or reduce an image are listed here:

- **You captured an image using a low magnification objective (e.g., 5x or below)** - In this case, enlarging the image adds pixels, which helps to smooth out the pixellation of high contrast, fine details. This makes it easier to view and interpret the structure of these details. Enlarging also makes large format prints appear smoother and less pixellated.
- **You want to save file space and resolution is not the primary concern** - In this case, reducing the image reduces the file size.

To resize an image, follow these steps:

1. From the Edit menu, select the resize option. The Resize Image window appears.
2. Do one of the following:
   - If you want to change the size of an image by a specific percentage, type in a value between 10% and 200%, or move the slider bar accordingly. The linear dimensions (width or height) of the image will change by this percentage factor.
   - If you want to specify the dimension in pixels, enter a number in either the Width or the Height box. The SPOT program automatically calculates the dimension not entered in order to maintain the proper aspect ratio.
3. Click on OK. The newly sized image replaces the original image on the screen.

*Note: If you want to create two differently sized images of an image for comparison purposes, use the Save As **[Ctrl A]** command to save the resized image with a different file name.*

Rotate Right

Rotate an image clockwise in 90 degree increments.

Rotate Left

Rotate an image counter-clockwise in 90 degree increments.

Flip Horizontal

Flip an image from right to left.

Flip Vertical

Flip an image from top to bottom.

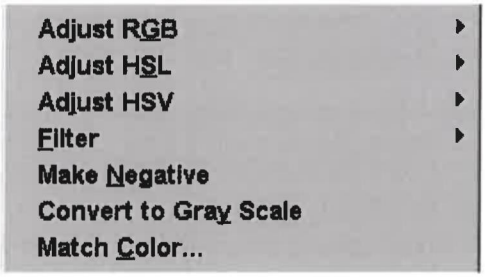
Crop

Cut out a portion of an image. To crop an image, follow these steps:

1. Press and hold the left mouse button.
2. Drag the cursor over the image until the dotted rectangle covers the area of the image that you want to crop.
3. From the Edit menu, select Crop. The Crop Area window displays the pixel coordinates of the rectangle that you selected. From this window you can fine tune the crop area by modifying the pixel coordinates. If you select the Crop option directly from the menu (i.e., without specifying a crop area), the Crop Area window displays the values for the entire image.
4. Click on OK to crop the image. The cropped image appears on-screen.

When you crop an unsaved image, the program automatically discards the area of the image outside the crop. To give yourself the opportunity to change the crop area, you should save the image before you crop it.

Image Quality

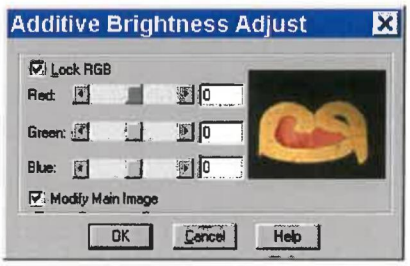


The image quality editing options provide you with a wide array of powerful and easy to use tools for changing the appearance of an image after it has been captured. These tools enable you to enhance the appearance of an image in ways that are not possible with conventional photography.

Before using the SPOT editing features, review the following two sections:

- **Functional Features** – This section explains how thumbnail images work in the SPOT image editing windows.
- **Image Editing and the Concept of Color Space** – This section introduces the concept of color space and describes how it applies to the SPOT software.

Functional Features



With the exception of Make Negative and Convert to Gray Scale, all of the image quality editing options utilize *thumbnail* images to preview the edits being made to the main image. As illustrated at right, a thumbnail of the image capture appears in the adjustment window, and the **Modify Main Image** box is checked as the default.

- **If you leave the box selected...** As you make editing adjustments, the changes appear in both the main image and the thumbnail.
- **If you deselect the box...** As you make editing adjustments, the changes appear only in the thumbnail image. After you click on OK, the changes appear in the main image.

Properly utilized, thumbnail images can be very helpful as part of the image editing process. Consider the following:

- ***You can use thumbnails to speed the editing process.*** The SPOT software often cannot update all the pixels in the main image as quickly as you can adjust the edit controls. In cases where the thumbnail image is sufficient to judge the effect of the edits, you can save time by deselecting **Modify Main Image**, so that the main image is updated only after you close the editing window. Deselecting **Modify Main Image** also makes the editing feel more "real time."

- ***The thumbnail can be used to display a smaller, magnified section of an image.*** When you select a specific image area (i.e., by dragging the cursor) before selecting an edit function, only the selected area appears in the thumbnail. This is useful for Sharpen, an edit function that affects only the fine structure of an image. This is also useful when you are analyzing histograms, and want the histogram to represent only the section of the image that interests you.

Image Editing and the Concept of Color Space

A color space is a coordinate system that is used to mathematically represent color. In order to satisfy the imaging requirements of different microscopy techniques, the SPOT program allows you to adjust brightness, color, and contrast in three color spaces: RGB, HSL, and HSV.

The color space options and their suggested uses are as follows:

- **Adjust RGB** (Red Green Blue) – Use for monochrome images
- **Adjust HSL** (Hue Saturation Luminance) – Use for all color images except fluorescence
- **Adjust HSV** (Hue Saturation Value) – Use for fluorescence images

Each color space is described below.

RGB The RGB color space operates by assigning a red, green, and blue value to each pixel in an image. Because red, green and blue are the primary colors for emissive light, this is the method that color computer monitors use to display images. However, the RGB color space is not intuitive to users.

HSL The HSL color space is a more intuitive model based on the three attributes that are common to all colors: hue, saturation, and luminance. Instead of assigning a red, blue, and green value to each pixel, the HSL color space assigns a value for each color attribute (e.g., hue, saturation, and luminance). These attributes are defined as follows:

- **Hue** – Hue is the designation for the actual color, as distinguished from others in the color spectrum. Hue is derived from a color wheel and is expressed in the color's angular location (in degrees) on the wheel. (e.g., +180° to –180° for the SPOT software).

- **Saturation** – Saturation is the proportion of perceived pure hue in the color. Saturation (in the SPOT software) is measured on a numerical scale of 0 to 100, where zero is pure gray and 100 is pure hue (e.g., day glow).

- **Luminance** – Luminance is the relative brightness or darkness of a hue. Luminance (in the SPOT software) is measured on a numerical scale of 0 to 100. A saturated color starts out as black at a luminance of 0, reaches full brightness as a saturated color at a luminance of 50, and then transitions through lighter pastel shades to white at a luminance of 100. This mimics the human perception of increasing brightness for images that are illuminated with light that contains a broad spectrum of wavelengths.

(cont.)

HSV The HSV color space was added to the SPOT software to optimize fluorescence image editing. Both the RGB and the HSL color spaces are problematic when used to edit color fluorescence images. Increasing gamma in RGB can cause dimmer sections of the image to lose saturation (look "washed out") and change hue. Increasing gamma or brightness in HSL causes the brighter areas of an image to look unnaturally pastel or even white. The HSV color space avoids both problems.

The HSV color space is similar to the HSL color space in that it also uses the intuitive concepts of hue and saturation. However, instead of luminance for brightness, it uses Value. Value is like Luminance in that a value of 0 is black. Value differs from Luminance in that a Value of 100 (in the HSV color space) is the brightest version of the original hue whereas a Luminance of 100 (in the HSL color space) is always white. For example, a dim cherry red changes to a bright cherry red when its Value is increased from 30 to 100. When its Luminance is increased from 30 to 100, a dim cherry red turns white.

HSV works well for fluorescence images because the dyes in fluorescence images emit single wavelength light. This light is, by definition, highly saturated. Editing in the HSV color space ensures that all areas of the image retain their saturated colors. Thus, using the HSV color space, images can be modified without causing the bright areas to look unnaturally pastel or white.

Adjust RGB/Adjust HSL/Adjust HSV

The process by which the program applies edits to an image capture differs according to the color space that you select. The following table briefly describes the edit process in each of the three color spaces and lists the recommended usage for each color space.

Color Space	Editing Process	Recommended Usage
RGB	Adjustments are applied separately to the red, green, and blue pixel values.	The RGB option is ideal for monochrome images.
HSL	1. RGB pixel values are translated to *HSL* (hue, saturation, and luminance) values. 2. Adjustments are applied to the hue, saturation, or luminance. 3. The HSL values are translated back to the RGB pixels.	The HSL option is ideal for all color images except fluorescence images.
HSV	1. RGB pixel values are translated to *HSV* (hue, saturation, and value) values. 2. Adjustments are applied to the hue, saturation, or value. 3. The HSV values are translated back to the RGB pixels.	The HSV option is ideal for fluorescence images.

The Adjust RGB, Adjust HSL, and Adjust HSV options are each made up of several image-editing functions. The table below lists the parallel RGB, HSL, and HSV functions:

Adjust RGB	Adjust HSL	Adjust HSV
Adjust Brightness (additive)	Adjust Hue, Saturation, and Luminance	Adjust Hue, Saturation and Value
Adjust Brightness (multiplicative)	Adjust Luminance (multiplicative)	Adjust Value (multiplicative)
Adjust Contrast	Adjust Contrast	Adjust Contrast
Adjust Gamma	Adjust Gamma	Adjust Gamma
Stretch Bright and Dark Level	Stretch Bright and Dark Level	Stretch Bright and Dark Level
Adjust Histogram	Adjust Histogram	Adjust Histogram

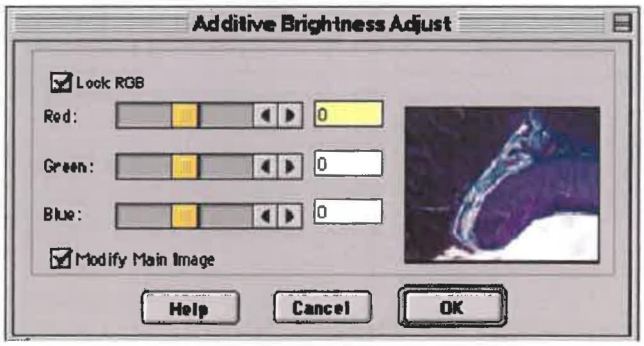
Many of the Adjust edit functions are duplicated in all three color spaces. To avoid unnecessary repetition, we have grouped together discussions of edit functions that are the same in different color spaces. The color space associated with the option appears parenthetically in the heading.

Adjust Brightness (additive) (RGB)

Use Adjust Brightness (additive) to lighten or darken an entire image. This function adds or subtracts the specified number(s) from the red, green, and blue values for every pixel in the image. Because this is an additive function, it affects bright and dark areas equally. You can change an image's brightness either by adjusting all three values (e.g., red, green, and blue) together, or adjusting each value individually.
To Adjust Brightness (additive), follow these steps:

1. From the Edit menu, select Adjust RGB and Adjust Brightness (additive). The Additive Brightness Adjust window appears, with a thumbnail preview of the open image.

   The Lock RGB and Modify Main Image boxes are checked as defaults. To adjust the RGB values individually, deselect the Lock RGB box.



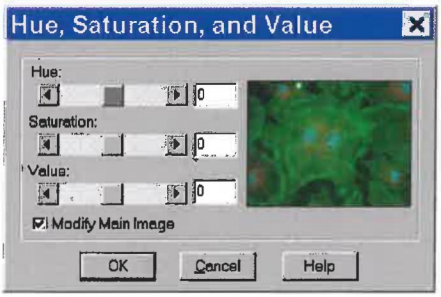
2. Change the brightness of the image.
   - To modify an image's brightness equally for red, green, and blue values, ensure that the Lock RGB box is checked, and either enter a number in any one of the three boxes, or use one of the slider bars to specify a number between -255 and 255.
   - To modify an image's brightness by specifying separate red, green, and blue values, deselect the Lock RGB box, and enter a value for each color.
3. Click on OK. The new image appears on-screen.

Adjust Hue, Saturation, and Luminance (HSL)
Adjust Hue, Saturation, and Value (HSV)

Use the Adjust Hue, Saturation, and Luminance and the Adjust Hue, Saturation, and Value options to make additive changes to the HSL or HSV values in an image. This process converts the RGB values for each pixel in the image to HSL or HSV values. After you make the adjustments, the program converts the HSL/HSV values back to RGB color space values.

To change the HSL or HSV values for an image, follow these steps:

1. From the Edit menu, select Adjust HSL, Adjust Hue, Saturation, and Luminance or Adjust HSV, Adjust Hue, Saturation, and Value:



2. Either type in the HSL/HSV values, or use the slider bars to select the values. Each value uses its own scale, and must be entered separately.
   - **Hue** - Enter an angle value between -180° and 180°. This number represents the amount that the color wheel is rotated for each pixel in the image.
   - **Saturation** - Enter a number between -100 and 100. This number is added to the saturation value (e.g., a number between 0 - pure gray and 100 - day glow) of each pixel in the image.
   - **Luminance or Value** - Enter a number between -100 and 100. This number is added to the Luminance/Value value (e.g., a number between 0 - pure black and 100 - pure white for Luminance/brightest version of hue if Value) of each pixel in the image.

Note: The scales used to measure HSL/HSV values are not standardized. Thus, the scales used by the SPOT software might differ from those of other programs.

3. Click on OK. The new image appears on-screen.

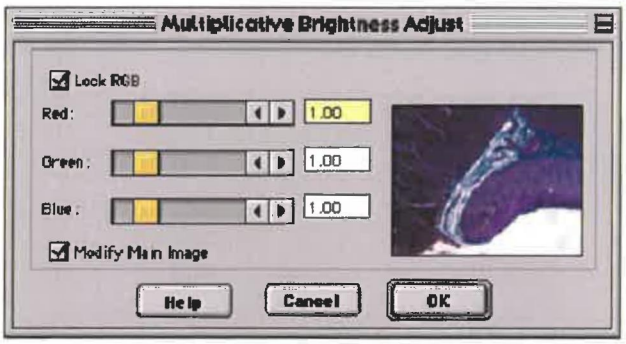
Adjust Brightness (multiplicative) (RGB)

Use Adjust Brightness (multiplicative) to make the bright areas of an image brighter or darker. This function multiplies the red, green, and blue values for every pixel in the image by the number(s) that you specify. Because this option is multiplicative, it affects bright areas more than dark areas: black areas tend to stay black while lighter areas get brighter. Like the Adjust Brightness (additive) option, the multiplicative option enables you to either adjust the RGB values together or as individual units.

To use the Adjust Brightness (multiplicative) function, follow these steps:

1. From the Edit menu, select **Adjust RGB > Adjust Brightness (multiplicative)**. The Multiplicative Brightness Adjust window appears, with a thumbnail preview of the open image.

   The Lock RGB and Modify Main Image boxes are checked as defaults. To adjust the RGB values individually, deselect the Lock RGB box.



2. Change the brightness of the image.

   – To modify an image's brightness equally for red, green, and blue values, ensure that the Lock RGB box is checked, and either enter a number in any one of the three boxes, or use one of the slider bars to specify a number between 0 (darkest) and 5 (brightest).

   – To modify an image's brightness by specifying separate red, green, and blue values, deselect the Lock RGB box, and enter a value for each color.

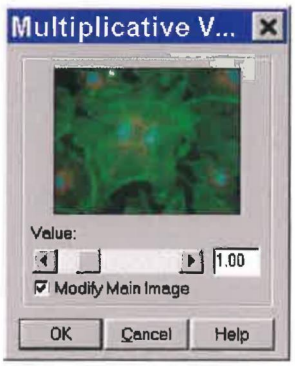
3. Click on OK. The new image appears on-screen.

Adjust Luminance (multiplicative) (HSL)
Adjust Value (multiplicative) (HSV)

Adjust Luminance (multiplicative) and Adjust Value (multiplicative) make the bright areas of an image brighter or darker when editing in the HSL and HSV color spaces. These functions multiply the Luminance or Value values for every pixel in the image by the number that you specify. Because these options are multiplicative, they affect bright areas more than dark areas.

To Adjust Luminance (multiplicative) (HSL) or Adjust Value (multiplicative) (HSV), follow these steps:

1. From the Edit menu, select Adjust HSL, Adjust Luminance (multiplicative) or Adjust HSV, Adjust Value (multiplicative):



The Modify Main Image box is checked as a default.

2. Change the Luminance or Value of the image. Use the slider bar to specify a number between 0 (darkest) and 5 (brightest).
3. Click on OK. The new image appears on-screen.

Adjust Contrast (RGB)

Use Adjust Contrast to exaggerate or subdue the differences between the bright and dark areas in an image. The Adjust Contrast option, when accessed from the Adjust RGB menu item, enables you to make adjustments to the red, green, and blue values either together, or as individual units.

To adjust the contrast in an image, follow these steps:

1. From the Edit menu, select Adjust RGB and Adjust Contrast. The Contrast Adjust window appears.

   The Lock RGB and Modify Main Image boxes are checked as defaults. To adjust the RGB values individually, deselect the Lock RGB box.

2. Change the contrast of the image.

   - To change the contrast equally for red, green, and blue values, ensure that the Lock RGB box is checked, and either enter a number in any one of the three boxes, or use a slider bar to specify a number between 0 (pure gray - no contrast) and 5 (maximum contrast).
   - To modify an image's contrast by specifying separate red, green, and blue values, deselect the Lock RGB box, and enter a value for each color.

3. Click on OK. The new image appears on-screen.

Adjust Contrast (HSL)
Adjust Contrast (HSV)

The Adjust Contrast (HSL) and Adjust Contrast (HSV) options, like the Adjust Contrast (RGB) option, exaggerate or subdue the differences between the bright and dark areas in an image. Unlike the Adjust Contrast (RGB) process, the Adjust Contrast (HSL/HSV) process works only on the Luminance/Value values for an image as opposed to the specific red, green, and blue values.

To use Adjust Contrast (HSL/HSV), follow these steps:

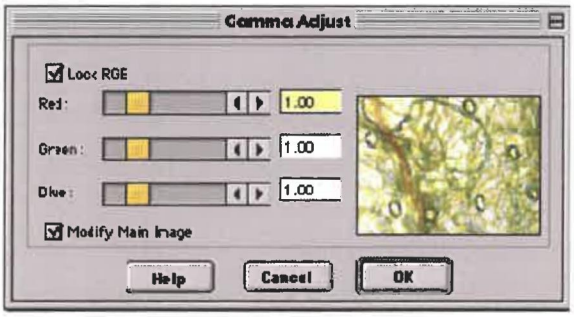
1. From the Edit menu, select Adjust HSL and Adjust Contrast or Adjust HSV and Adjust Contrast.

The Modify Main Image box is checked as a default.

2. Enter a value between .1 and 4, or use the slider bar to select a value. The thumbnail changes as you adjust the value.
   - To exaggerate the contrast of an image, enter a value above one (the base value).
   - To subdue the contrast of an image, enter a value below one (the base value).
3. Click on OK. The new image appears on-screen.

Adjust Gamma (RGB)

Gamma Adjust smoothly lightens darker areas of an image without burning out bright areas or lightening black areas. This is especially useful for editing images with a wide range of brightness values (dynamic range). Gamma adjustment corrects an image by creating a new version of the original. Refer to Ch. 5 – Preparing to Take a Picture for a more detailed explanation of gamma adjustment.



To adjust gamma, follow these steps:

1. From the Edit menu, select Adjust RGB and Adjust Gamma. The Gamma Adjust window appears. The Lock RGB and Modify Main Image boxes are checked as defaults.
2. Gamma adjust the image.
   - To adjust the gamma equally for red, green, and blue values, ensure that the Lock RGB box is checked, and either enter a number in any one of the three boxes, or use a slider bar to specify a number between .1 and 4.
   - To gamma adjust an image by specifying separate red, green, and blue values, deselect the Lock RGB box, and enter a value for each color.

Note: The ability to deselect the Lock RGB box and enter specific RGB values distinguishes the use of the Adjust Gamma menu option from the Gamma Adjust option on the Image Setup window (see Ch. 5 – Preparing to Take a Picture).

3. Click on OK. The new image appears on screen.

Adjust Gamma (HSL)
Adjust Gamma (HSV)

Like the Adjust Gamma (RGB) option, the Adjust Gamma (HSL) and Adjust Gamma (HSV) options allow you to smoothly lighten darker areas of an image without burning out bright areas or lightening black areas. This is useful when modifying images with a wide *dynamic range*, meaning the range of brightness values recorded by the CCD chip. Unlike the Adjust Gamma (RGB) option, Adjust Gamma (HSL) and Adjust Gamma (HSV) work only on the luminance or value values (respectively) for an image as opposed to the specific red, green, and blue values. Refer to Ch. 5 – Preparing to Take a Picture for a more detailed explanation of gamma adjustment.

To Adjust Gamma (HSL/HSV), follow these steps:

1. From the Edit menu, select Adjust HSL, Adjust Gamma or Adjust HSV, Adjust Gamma.

2. Enter a value between .1 and 4, or use the slider bar to select a value. The thumbnail preview of the image changes as you adjust the value.
   - To lighten the mid-tones of an image, enter a gamma value above one.
   - To darken the mid-tones of an image, enter a gamma value below one.
3. Click on OK. The new image appears on-screen.

Stretch Bright and Dark Level (RGB)

The Stretch Bright and Dark Level (RGB) option stretches the darkest level in an image to 0 (black) and the brightest level to full scale. Full scale is 255 for 8 bit monochrome and 24 bit RGB images, and 4,095 for 12 bit monochrome and 36 bit RGB images.

Stretching the bright and dark level (RGB) for a 24 bit RGB image with a range of brightness values between 50 and 150 has the following effects:

- The lowest value pixels (50) are stretched down to 0.
- The highest value pixels (150) are stretched up to 255.

This one-step process provides a quick and easy way to increase image contrast in low contrast specimens. To use Stretch Bright and Dark Level (RGB), select Adjust RGB, Stretch Bright and Dark Level from the Edit menu. The picture automatically adjusts itself.

Stretch Bright and Dark Level (HSL)
Stretch Bright and Dark Level (HSV)

Like Stretch Bright and Dark Level (RGB), the Stretch Bright and Dark Level options in the HSL and HSV color spaces stretch the darkest luminance or value level in an image to 0 (black) and the brightest luminance or value level to 100 (pure white if Luminance/brightest version of hue if Value). Using the HSL or HSV (for fluorescence images) color space to stretch images, as opposed to the RGB color space, reduces the potential for washed out images and color shifts.

To stretch bright and dark level HSL or HSV, select Adjust HSL, Stretch Bright and Dark Level or Adjust HSV, Stretch Bright and Dark Level from the Edit menu. The picture automatically adjusts itself.

Adjust Histogram (RGB)

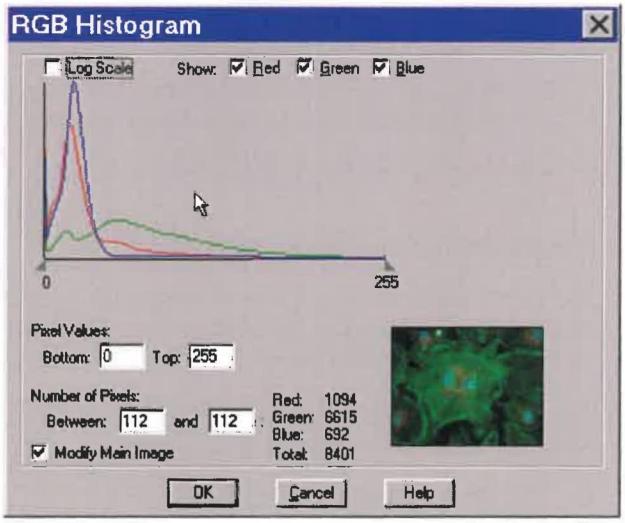
A *histogram* is a graphical representation of the number of pixels at each brightness level in an image. SPOT's histogram displays the red, green, and blue histograms on the same graph. It serves two main purposes:

- It is a point of reference for examining the distribution of brightness levels for the red, green, and blue components in an image.
- It can be used to reset the zero and full scale points for an image. This is known as *stretching*. Like the aforementioned Stretch Bright and Dark Level function, the Adjust Histogram option enables you to stretch the darker areas of your image to pure black and at the same time lighten the brighter areas of the image.

Note: Full scale for 8 bit monochrome and 24 bit RGB images is 255. Full scale for 12 bit monochrome and 36 bit RGB images is 4,095.

To adjust an RGB histogram, follow these steps:

1. From the Edit menu, select Adjust RGB, Adjust Histogram. The RGB Histogram window appears. Set the display parameters for the histogram:
   - Determine the color or colors that you want to display brightness values for. As a default, the histogram displays all three values. To display a different combination of colors, deselect the Show box for the unwanted color(s).
   - To display the histogram according to a logarithmic scale (as opposed to a linear scale), check the Log Scale box. This makes the vertical axis more sensitive to small numbers and compresses the range for larger numbers.



From the RGB Histogram screen you have three viewing/editing options.

- ***Examine the number of pixels at each brightness level.***
  Do one of the following:
  - Move the cursor along the graph to display the number of pixels with red, green, and blue values at each brightness level and the total number of pixels at that level.
  - Type the same brightness level into both boxes under Number of Pixels. The histogram readouts display the number of pixels with red, green, and blue values at that brightness level, as well as the total number of pixels at that level.
- ***Examine the number of pixels between two brightness levels.***
  Do one of the following:
  - Position the cursor over one brightness level on the graph, and left click and drag the cursor to the second brightness level. The histogram readouts display the number of pixels with red, green, and blue values between the two brightness levels as well as the total number of pixels.
  - Type the two brightness levels into the boxes under Number of Pixels. The histogram readouts display the number of pixels with red, green, and blue values between the two brightness levels, as well as the total number of pixels.
- ***Stretch the image.***
  Follow these steps

  a) Either drag the left triangle (on the X axis) to the brightness level that you want to define as zero, or enter the value in the **Bottom** box. All red, green, and blue values that are less than or equal to this level are now reset to zero.

  b) Either drag the right triangle (on the X axis) to the brightness level that you want to define as full scale, or type in the value in the **Top** box. All red, green, and blue values that are greater than or equal to this level are now reset to full scale.

  The program automatically multiplies all red, green, and blue values between the new zero point and full scale values by the factor necessary to make the new full scale setting equal to actual full scale after the new zero point setting (if you have defined one) has been subtracted.

  c) Click on OK to save the image with the new zero point (Bottom) and full scale (Top) values.

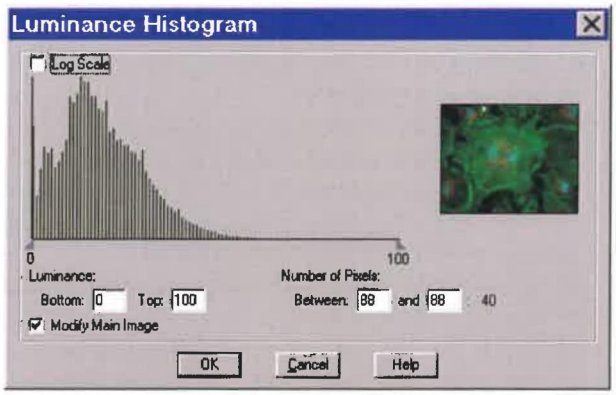
Adjust Histogram (HSL)
Adjust Histogram (HSV)

HSL and HSV histograms are identical to RGB histograms, except that instead of displaying the number of pixels for the RGB components, they display the number of pixels at each luminance or value level. As with the RGB histogram, HSL and HSV histograms are typically used for two purposes:

- Examining the distribution of luminance or value levels in an image.
- Resetting the zero and 100 % luminance/value points in an image. This is known as *stretching*. The Adjust Histogram (HSL) and Adjust Histogram (HSV) options allow you to stretch the darker areas of your image to pure black and lighten the brighter areas to either pure white, if using the HSL color space, or the brightest version of the hue, if using the HSV color space.

To Adjust Histogram (HSL) or Adjust Histogram (HSV), follow these steps:

1. From the Edit menu, select Adjust HSL, Adjust Histogram or Adjust HSV, Adjust Histogram. The Luminance Histogram or Value Histogram window appears.
2. If you want to display the image's histogram according to a logarithmic scale, check the Log Scale box. Checking the Log Scale box makes the vertical axis more sensitive to small numbers and compresses the range for larger numbers.



From the Luminance/Value Histogram window you have three viewing/editing options.

- ***Examine the number of pixels at each Luminance/Value level.***
  Do one of the following:
  - Move the cursor along the graph to display the number of pixels at each Luminance/Value level.
  - Type the same Luminance/Value level into both boxes under Number of Pixels. The histogram readouts display the number of pixels at that Luminance/Value level.
- ***Examine the number of pixels between two brightness levels.***
  Do one of the following:
  - Position the cursor over one luminance or value level on the graph, and left click and drag the cursor to the second Luminance/Value level. The histogram readouts display the number of pixels between the two levels.
  - Type the two Luminance/Value levels into the boxes under Number of Pixels. The histogram readouts display the number of pixels between the two Luminance/Value levels.
- ***Stretch the image.***
  Follow these steps:

  a) Either drag the left triangle (on the X axis) to the Luminance/Value level that you want to define as black (0 % Luminance/Value), or enter the Luminance/Value number in the **Bottom** box. All Luminance or Value values that are less than or equal to this level are now reset to black (0 % Luminance/Value).

  b) Either drag the right triangle (on the X axis) to the Luminance/Value level that you want to define as full scale (100 % Luminance/Value), or enter the Luminance/Value number in the **Top** box. All Luminance/Value values that are greater than or equal to this level are now reset to full scale (100 % luminance/value).

  The program automatically multiplies all Luminance/Value values between the new black and full scale values by the factor necessary to make the new full scale value equal to 100 % Luminance/Value after the new black value (if you have defined one) has been subtracted. The thumbnail sample image updates automatically so you can gauge the effect of your adjustments.

  c) Click on OK to save the image with the new black and white Luminance/Value values.

Filter

The Filter menu option contains four options for modifying images:

- Smooth
- Sharpen
- Unsharp Mask
- Filter Noise

Smooth

Use Smooth to smooth out grainy images. The smooth option works by lightening the darker side of contrast boundaries and darkening the lighter side. Because the Smooth option removes the high frequency details from an image, some fine image detail is also lost.

To smooth the appearance of an image, follow these steps:

1. From the Edit menu, select **Filter > Smooth**.

   To preview images on the thumbnail image only, deselect the Modify Main Image box.

2. Either use the slider bar to select a Strength value between one and 100, or enter the value in the box.

3. When you have determined a suitable value, click on OK. The smoothed image appears.

Sharpen

Use Sharpen to improve the appearance of soft or blurry looking pictures. The Sharpen option works by darkening the darker side of a contrast boundary and brightening the brighter side of a contrast boundary.

To sharpen the appearance of an image, follow these steps:

1. From the Edit menu, select **Filter > Sharpen**.

   To preview changes on the thumbnail image only, deselect the Modify Main Image box.

2. Either use the slider bar to select a Strength value between one and 100, or enter the value in the box.

3. When you have determined a suitable value, click on OK. The sharpened image appears.

Unsharp Mask

Use the Unsharp Mask filter to improve the appearance of an image's high frequency details. The Unsharp Mask option sharpens an image by subtracting a brightness-scaled, low-pass-filtered image from the original image.

To apply the Unsharp Mask filter, follow these steps:

2. From the Edit menu, select **Filter > Unsharp Mask.**

   To preview changes on the thumbnail image only, deselect the Modify Main Image box.

3. Either use the slider bar to select a Strength value between one and 100, or enter the value in the box.

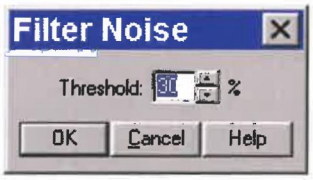
4. When you have determined a suitable value, click on OK. The sharpened image appears.

Filter Noise

The Noise Filter corrects for the effects of electrical or thermal noise. When capturing dim images with lengthy exposure times, the noise filter acts as a "virtual cooler" for the Insight camera, eliminating "hot pixels." The Noise Filter checks each pixel on the CCD chip as described below:

1. The program compares the value of each red pixel with the values of the eight neighboring red pixels.
2. If none of the eight neighboring red pixels has a value within ±15 % (i.e., the threshold value that the SPOT program uses) of the value for the red pixel being checked, the value for the red pixel being checked is replaced with the average value of the eight neighboring red pixels.
3. The program repeats this procedure for the values of the green and blue pixels being checked.

To filter noise, follow these steps:



1. From the Edit menu, select **Filter > Filter Noise**.
2. Enter a value between **10%** and **100%**. The Threshold value is set to a default of 50%. Refer to the procedure described at the start of the Filter Noise section for an explanation of the Threshold value.
3. Click on OK. The filtered image appears on-screen.

Note: Image setups can be defined to automatically use the noise filter when capturing an image. Refer to Ch. 5 – Preparing to Take a Picture for details.

Make Negative

Use Make Negative to make a color negative (with complimentary colors) of an image. From the Edit menu, select Make Negative. The Program automatically converts the image to a negative.

Convert to Gray Scale

Use Convert Gray Scale to convert images from color to gray scale. The Convert to Gray Scale option works only with color (24 bit and 36 bit) images.

Insight B/W	The Convert to Gray Scale option is available only for RGB images that have been opened with the SPOT software/Insight Color camera. For monochrome image captures, the Convert to Gray Scale option is unavailable.

From the Edit menu, select the Convert to Gray Scale option. The program converts the color image to a gray scale image.

Set Palette (monochrome images)

The Set Palette option enables you to apply a specific color palette to a captured monochrome image. There are four pre-defined color options: **Grayscale**, **Red**, **Green**, and **Blue**.

To define a custom palette, use the **Palettes** option (Setup>Palettes). Refer to Ch. 4 – The Advanced Program Mode for more details. **Insight B/W** users can also apply palettes as part of the image capture process using the **Palette** option on the Image Setup window.

Match Color

The Match Color feature is typically used in two situations:

- You want to change the colors of an image on the screen to match the way that you think the specimen looks.
- You want to set the white balance for pictures taken with a microscopic technique that never has white in its images. A prime example of this situation is fluorescence microscopy.

Note: The Match Color white balance adjustment can be used as an alternative to the Compute White Balance Values option.

To use the Match Color feature, follow these steps:

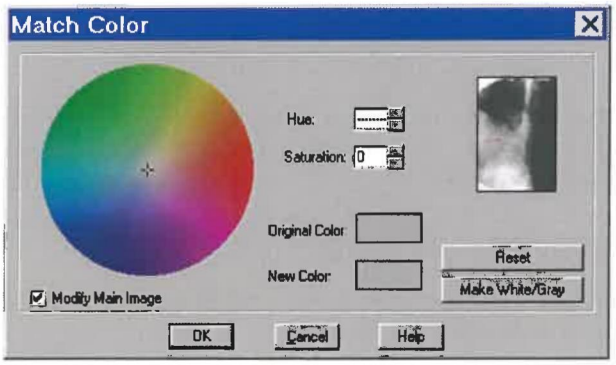
1. From the Edit menu, select Match Color.
2. Click on OK. The cursor becomes an eye-dropper.
3. Use the mouse to move the eye-dropper to the pixel location of the color that you want to match. Refer to the status bar at the bottom of the window to ensure that each of the R, G, and B values are less than full scale (255 for 24 bit RGB and 4,095 for 36 bit RGB).

Note: If you select a pixel with a red, green and/or blue value that is saturated at full scale, the SPOT program prompts with a warning message indicating that you need to choose a pixel with RGB values less than 255 (24 bit RGB) or 4,095 (36 bit RGB). Saturated pixels do not provide a good reference point for correctly determining the RGB ratios needed for this function.

4. Click on the pixel. The Match Color window appears.

Note: The Set White Balance button (not shown above) appears only for image captures that have not yet been saved and closed.

Insight B/W Insight B/W camera users will not see the Set White Balance button.



The window displays information about the pixel that you selected:

- **Hue** – This the actual color, as distinguished from others in the color spectrum. Hue is derived from a color wheel and is expressed in the color's angular location (e.g., 0 to 360 degrees).
- **Saturation** - The proportion of perceived pure hue in the color. Saturation (in the SPOT software) is measured on a numerical scale of 0 to 100, where zero equals pure gray and 100 equals pure hue (i.e., "day glow").
- **Original Color** – A sample "swatch" of the color that you selected. The cross hairs on the color wheel indicate the relative position of the color.

The **Modify Main Image** box is checked as a default.

5. Set the new color. This can be done in one of two ways, depending on whether the image should contain white:
   - If you are trying to make the selected pixel in your image appear as white (or gray if the pixel is darker), click on the Make White/Gray button.
   - If you are working with an image that does not have any white, modify the Hue and Saturation values so that the New Color "swatch" shows up as the color that you want. You can do this either by entering specific Hue and Saturation values, or dragging the cross-hairs on the color wheel to the desired location.

At this point there are three options:

- Click on **OK** to save the image with the new color rendition.
- Click on **Reset** to return the image to the original color rendition.
- For images that have not yet been saved and closed, click on **Set White Balance** to set the values so that colors are rendered correctly for future image captures. When you click on Set White Balance, a dialog box displays the white balance values. As a default, the values are saved to the current setup. If desired, you can select a different Setup and then click on OK.

6. If you reset the White Balance, you can now recapture the image with the new settings.

Customizing Images

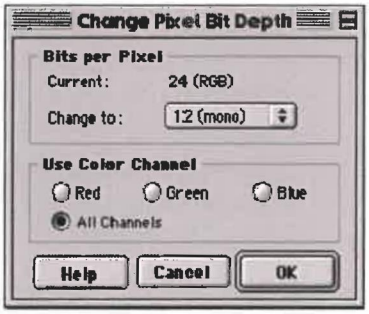
In addition to the image editing features described in this chapter, the SPOT software offers several options for customizing images.

- **Change Pixel Bit Depth** – Change from one bit depth to any of the other three options without having to recapture an image.
- **Subtract Image** – Subtract an image from a selected open image.
- **Merge Images** –Merge two or three images, assigning red, green, or blue to each image. The Merge Images feature is mainly used to combine two or three monochrome images into an artificially colored composite image.

Change Pixel Bit Depth

Use Change Pixel Bit Depth to change the bit depth of the currently open image. To change the pixel bit depth for an image, follow these steps:

1. From the Edit menu, select the Change Pixel Bit Depth option. The Change Pixel Bit Depth window displays the current Pixel Bit Depth for image.
2. Select the pixel bit depth that you want to change the image to.
   - Changing from one monochrome bit depth to another (8 bit to 12 bit) or from one RGB bit depth to another (24 bit to 36 bit) does not require the addition of any color channeling information. In this case, select the new pixel bit depth and click on OK.



CAUTION!

If you are changing an image to either 12 bit mono-chrome or 36 bit RGB, you must save the image in TIFF format before you can close it. Refer to step four for further instructions.

– Changing from an RGB (24 bit or 36 bit) color image to a monochrome image (8 bit or 12 bit), requires the entry of color channeling information. When you select the new pixel bit depth, the color channeling information appears.

  Select the color channel(s) that you want to use. Select either a single color or the All Channels option, which is the default.

  - If you select a single color channel, the program uses only the values for that color (in the original image) when it creates the monochrome image.
  - If you select All Channels, the program uses a mathematical algorithm to combine the red, green, and blue values from the original image when it creates the monochrome image.

CAUTION!

If you are changing an image to either 12 bit monochrome or 36 bit RGB, you must save the image in TIFF format before you can close it. Refer to step four for further instructions.

3. Click on OK to save the image with the new pixel bit depth.

4. If you changed the original pixel bit depth to either 12 bit or 36 bit, follow these steps before trying to save the image to disk:

   a) From the File menu, select the Save As option. The Save As window appears. The Save as type dialog box displays **TIFF Uncomp (12-bit/channel) (TIFF)**.

   b) The Save as type drop-down box has two options:

   - **TIFF Uncomp (12-bit/channel) (TIFF)** – Select the 12 bit per channel option if you want to save the image as 36 bpp.
   - **TIFF Uncomp (16-bit/channel) (TIFF)** – Select the 16 bit per channel option if you want to save the image as 48 bpp. This allows you to edit images with Adobe Photoshop, which cannot read 36 bpp images.

7. Enter the desired file name and location, and click on the Save button.

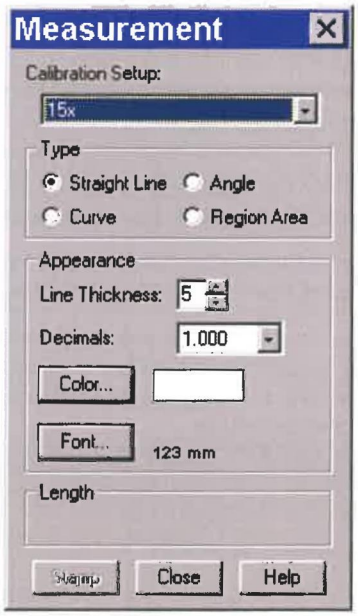
6. Enter the size of the calibration mark. You can modify the size either by entering the number in the Size box, or by using the cursor to stretch the on-screen calibration bar. To adjust the size of the calibration mark, move the cursor to the end of the calibration bar line. The standard cursor changes to a two-headed arrow. Drag the end of the bar to adjust the length. The calibration mark number changes to reflect the new length.
7. Click on Stamp to incorporate the calibration mark into the image.

Add Measurement

The Add Measurement option enables you to incorporate measurements into an image capture, including line, area, and angle measurements.

To add a measurement, follow these steps:

1. If you have not already calibrated the objectives on your microscope, do so. Refer to Ch. 4 – The Advanced Program Mode for details.
2. From the Edit menu, select Add Measurement. The Measurement window appears.
3. From the **Calibration Setup** drop-down list, select the setup (e.g.,15x) for the objective that you are using.
4. Check the type of measurement that you want.
   - **Straight Line** – Point to point distance measurement
   - **Curve** – Length of a freehand line
   - **Angle** – Angle measurement
   - **Region Area** - Area of a freehand outline
5. Move the cross-hair cursor to the point in the image capture where you want to begin the measurement.
6. Single click to activate the drawing function. ***Do not hold the mouse button down between the measurement points.***
   - For a **Straight Line**, move the cross-hair cursor to the end point of the measurement. Single click to deactivate the drawing function and display the length of the line.
   - For a **Curve**, use the cross-hair cursor to trace a freehand line on an image. Single click to deactivate the drawing function and display the length of the freehand line.
   - For an **Angle**, use the cross-hair cursor to specify the three points that define the angle. Single click to mark the end point of the first side. Move the cross-hairs to the vertex, and single click again. Move the cross-hairs to the end point of the second side, and single click. This last click deactivates the drawing function and displays the angle measurement.
   - For a **Region Area**, use the cross-hair cursor to trace a freehand outline around the area that you want to measure. Single click to deactivate the drawing function and display the measurement. If the area is not completely enclosed by the outline, the SPOT program automatically completes the path by connecting the end point to the starting point with a straight line.



When you complete a measurement, the drawing function is deactivated, and the cursor changes back to a standard arrow. At this point you can:

- Re-position the measurement number. When you position the cursor over the number, it changes to a four point arrow. Click and drag on the number to move it to a new location.
- Erase the last measurement. Double-click anywhere on the image to erase the previous measurement. The cross-hair cursor re-appears for you to draw another measurement

After you have completed steps one through six, you can modify the measurement as needed, using the Appearance options:

- **Line Thickness** – Select a line thickness between 1 (thinnest) and 5 (thickest).
- **Decimals** – Select the number of decimals that appear in the measurement. Choose from a range of zero decimals (1) to five decimals (1.00000)
- **Color** – Click on the Color button to define the color of the measurement. You can either choose from a palette of basic colors or define a custom color to provide the best display. If you are editing a monochrome image, you can select either black or white.
- **Font** – Define the font, point size, and appearance.

7. Click on **Stamp** to incorporate the measurement into the image. The cross-hair cursor reappears for you to add additional measurements to the image.

Annotation - Custom Image Annotation

Image annotation is useful when you want to prepare captures for formal presentation, or inclusion in an article or reference work. Using SPOT's image annotation features, you can:

- Add text to an image.
- Use an arrow, line, rectangle, or ellipse to emphasize a particular area in an image.
- Add a graphic stamp such as a company logo to an image.
- Add freehand lines to an image.

When you select the Annotate option on the Edit menu, several changes take place in the SPOT image editing environment:

- The **Edit** menu options change to reflect annotation editing, as opposed to the image editing discussed earlier in this chapter.
- The **Object** menu appears with options for inserting drawing objects, text, images, and image related information onto an image. Cursor functionality in SPOT reports varies according to which **Object** menu option is selected.
  - If **None** is selected, the cursor is a an editing tool for selecting, sizing, and positioning objects. When creating new reports, **None** is the default option.
  - If a report object is selected, the cursor changes to a cross-hair icon for placing new report objects on the grid.
- The **View** menu displays two options:
  - **Show Pan Window** – Refer to the beginning of this chapter for a description of this function.
  - **Show Tools** – This option displays the Tools dialog box, which contains shortcuts for inserting annotation drawing objects. Refer to the Adding Annotation Objects to SPOT Images section (below) for more details on using the Tools dialog box,

- The **Attributes** menu appears with options for specifying annotation qualities.
- The **Done!** menu (Windows)/**Mode** menu (Mac) allow you to switch back from annotation mode to image capture/edit mode.
  - **Windows** – The **Done!** menu has only one option, **End Annotation Mode**. When you select this option, the SPOT program prompts you to merge the changes into the image.
  - **Mac** – The **Mode** menu has only one option, **Exit Annotation Mode**. When you select this option, the SPOT program prompts you to merge the changes into the image.

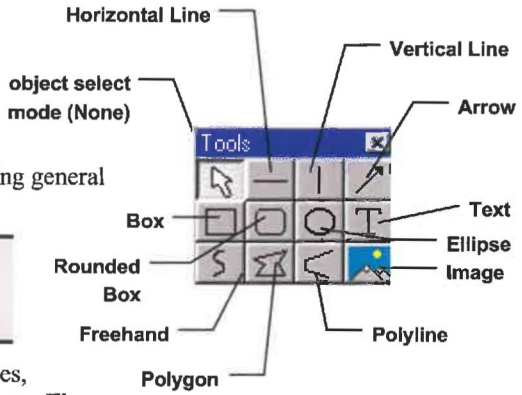
CAUTION!

If you select Yes after clicking on Done! The annotations become a permanent part of the image. If you want to preserve the original image, save the original image to a file. After annotating it, use Save As to save the annotated version to a different file.

Adding Annotation Objects to SPOT Images

Annotation objects can be added to images in one of two ways:

- Through the **Object** menu.
- Through the Tools dialog box. To access the Tools dialog box, select the **Show Tools** option on the View menu. The Tools dialog box, as illustrated below, displays shortcut buttons to the eleven drawing objects on the Object menu:



Before discussing the individual objects, review the following general procedure for inserting an annotation object into an image:

Note: In some cases, the procedure varies to fit the object's characteristics. The specifics are noted in the descriptions of the individual objects.

1. From the Object menu, select the type of item (i.e., Lines, Connected Lines, etc…) that you want to add to the image. The cursor's functionality varies according to whether a report object type (i.e., line, text) is selected:.
   - If a report object type is selected, the cursor changers to a cross-hair icon that is used to add new report objects to the grid.
   - If **None** is selected, the cursor is a an editing tool that is used to select and position objects.
2. Use the mouse to drag the cross-hair to the location where you want to insert the object.
3. Left-click at the insertion point.
   - If you selected a line, box, or other shape, hold down the mouse button and drag the object to the desired size.
   - If you selected any of the other objects, the object will either appear (e.g., Image Time) or the program will prompt you to enter the associated information (e.g., Text, Image Memo, etc…).
4. After you have added the object, there are three options:
   - Add another object of the same type, as described in step 3.
   - Add a different type of object - select from the Object menu options.
   - Resize, reposition, or edit an object. To do so, select **None** from the Object menu.
     - **Resize** - Select (click on) the object and drag the handles in the direction of the resize.
     - **Reposition** – Left click and drag the object the new area.
     - **Edit** – Select the object and choose an option from either the Edit menu or the Attributes menu. You can also edit an object by **right clicking** on it.

The following sections describe each of the annotation objects.

None

Select **None** when you want to use the cursor to edit and position the objects.

Lines

Unconstrained Line - Add a straight line annotation to the image

Horizontal Line - Add a horizontal line annotation to the image.

Vertical Line - Add a vertical line annotation to the image.

Arrow – Add an arrow to the image. Either select Lines>Arrow from the Object menu, or click on the Tools box button. Left click on the image and drag toward the area that the arrow head will point to.

Connected Lines

Polygon – Add a closed polygon to an image. Either select Polygon from the Object menu, or click on the Tools box button. Left click and release, and then drag the mouse to create a side. Left click again to start a new side. Double click to finish the polygon. The program automatically adds any additional lines needed to close the figure.

Polyline – Add an open polygon to an image. Either select Polyline from the Object menu, or click on the Tools box button. Left click and release, and then drag the mouse to create a side. Left click again to start a new side. Double click to finish the polyline.

Freehand - Add a freehand line to the image

Shapes

Rectangular Box – Add a square cornered box to the image.

Rounded Box - Add a round cornered box to the image.

Ellipse – Add an elliptical object to the image. Ellipses are drawn inside of the rectangular area that you define. Ellipses touch the top, bottom, right and left sides of this rectangle.

Either select Ellipse from the Object menu, or click on the Tools box button. To draw an ellipse, left click at the point where you want to position one vertex of the rectangle and drag the cursor to the point of the opposite vertex.

Images

Stamp – Insert a selected bitmap image onto the image capture. From the Object menu, select Stamp. The last selected bitmap appears on your image. To select a new bitmap image to insert, choose the Stamp Bitmap option from the Attributes menu.

Image – Add an image.

1. From the Object menu, select Image.
2. Left click at the point on the display grid where you want to insert the Image box, and holding down the mouse button, drag the cursor to create the desired shape and size for the image (it can also be resized/re-positioned at a later point). When you release the mouse button, the Select Image dialog box appears.
3. Specify the file source for the image:
   - **Currently Open Image** – Add a currently open image . If you have one or more open images, SPOT lists the file name(s) and path(s) in the drop-down box. Select from the list.
   - **Image File –** Add an image from disk. Specify the file name and location either by entering the path in the Name box, or clicking on the selection box to the right.
   - **Database (Windows only)** – Add an image from a SPOT database. In order to insert a database image, the database must be open. Enter the Image ID for the file. If you do not know the database image ID, search the database – the display table indicates the Image ID for each file.

   Click on OK. The image appears on your report grid.

Because the Mac edition does not yet have database functionality, Mac users will not be able to use the Database option. Diagnostic Instruments provides free software updates via the Web to all SPOT camera owners. Check the Diagnostic Instruments web site (www.diaginc.com) for the most recent update. For details on finding and downloading the latest version of the software, refer to the **Free Software Updates** section in "About This Manual."

Text **Text** - Add text to the image.

1. Either select **Text>Text** from the Object menu, or click on the Tools box button.
2. Left click at the point where you want to insert the text. The Text window appears.
3. Enter the text.
4. Click on OK. The text box appears.
5. Resize the box as needed to display the text.

Image Date - Add a date to the image. This date defaults to the date that the image was captured. To change the date, double click on the box, and enter the desired date.

Image Time - Add the image capture time to the image. This time defaults to the time that the image was captured. To change the time, double click on the box, and enter a new time. The time that appears here varies according to whether the file was transferred across a network, and whether the file is a SPOT database image. Refer to the Image Date/Time section of this chapter for more details.

Image Title - Add a title to the image. The title automatically defaults to the file name and location of the open image, e.g., **C:\images\cells\fluor1**. To change the title, double click on the box and enter a new title.

Image Memo – Add a previously defined TIFF image memo. Click at the point where you want to insert the text box. To change the memo, double click on the box and modify the text as needed. Changing the text in the memo annotation does not affect a memo that is attached to the image file.

Image Sequence Index (sequence images) - Add the Sequence Index (number) to the image. This is the number of the image within the sequence – i.e., the Sequence Index for the first image in the sequence is "1," the second image "2," etc...

Image Sequence Elapsed Time (sequence images) – Add the elapsed time between the image being annotated and an image that you specify. After selecting **Object>Text>Image Sequence Elapsed Time**, the program prompts you to enter the **Reference Image Index Number**. If you specify a number that comes after the image being annotated, the elapsed time will be negative. For instance, if you are annotating image number five in a sequence of 10, and you specify a Reference Image... of eight, the elapsed time will be a negative value.

User Name – Add the user's name to the image. This defaults to the user who is logged on the computer.

Defining Annotation Attributes

All of the general style and appearance options can be accessed from the Attributes menu. Annotation attributes can be defined for an entire report or on an object by object basis (see the Adding Annotation Objects to SPOT Images section above).

- When an annotation object is selected, the attribute is defined for the selected object(s) only. In addition, when you right click on an object, the menu displays the attribute options for that object.
- When nothing is selected, the attribute is defined for all future, relevant objects.

The following table details each of the annotation attributes and the report objects that they affect.

Line Thickness

Determine line thickness for the following objects: **Line, Horizontal Line, Vertical Line, Arrow, Box, Rounded Box, Ellipse, Freehand, Polygon**, and **Polyline.**

1. From the Attributes menu, select Line Thickness.
2. Enter a Thickness value between **1** and **5** pixels.
3. Click on OK.

Colors

Define the foreground and background colors for all objects. Follow these steps:

1. From the Attributes menu, select Colors.
2. Define the **Text/Lines** color. This is the color of the text, the line, or the border around a text/image box. The default foreground color is black. To select a color click on Color.
3. The next window varies, depending on whether you are using a Windows or a Mac system.
   - Windows allows you to select from a palette of colors or enter specific HSL or RGB color values.
   - Mac OS allows you to select from a palette of colors, enter specific RGB, CMYK, HLS, or HSV values, and enter Internet specific colors.
4. Define the **Background/Fill** color. This is the color that appears behind text and in boxes. Lines and images do not display a background color.

Note: In order to define a background/fill color for a Box or a Rounded Box, a fill must be selected. Similarly, in order to define a background/fill color for a text box, the background (see Text Border, Justification, and Background below) must be set to Opaque.

Arrow Size and Direction

Specify the size of the arrow head and define the direction that the arrow points.

1. To specify the size of the arrow head, enter a value between **1** and **10**.
2. Specify the direction of the arrow:
   - To draw an arrow starting tail first and finishing with the point (head), select **Tail to Head**.
   - To draw an arrow starting point (head) first and finishing with the tail, select **Head to Tail**.
3. Click on OK.

The Arrow Size and Direction attribute is not available for Mac users. For Mac users, arrows are always drawn **Tail to Head**.

Image Border

Determine the line thickness for image borders. This option applies only to the **Stamp** and **Image** objects.

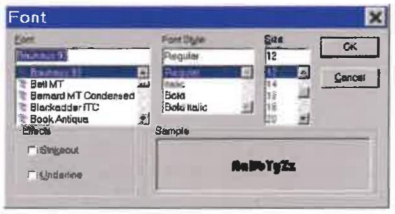
1. Select the object.
2. To create a border around the image, click on the Border box, and enter a **Thickness** value between **1** and **5** pixels..
3. Click on OK.

Box/Ellipse Fill

Define the appearance of the **Box**, **Rounded Box**, and **Ellipse** objects.

1. Specify whether you want a fill for the object.
   - If you do not want the box to be filled (i.e., you want a transparent box), click on OK.
   - If you want to specify a fill pattern for the box, check the Fill Box/Ellipse. The **Pattern** options appear.
2. Specify a pattern by clicking on one of the seven options.
3. Click on OK.

Text Font



Define the appearance of the text for the **Text**, **Image Date**, **Image Time**, **Image Title**, **Image Memo** objects.

1. Select a **Font**, a **Font Style**, and a point **Size**. The Sample box displays a preview of the font.
2. Define any Strikeout or Underline effects.
3. Click on OK.

Text Border, Justification, and Background

Define the appearance of the text boxes for the **Text**, **Image Date**, **Image Time**, **Image Title**, **Image Memo** objects.

1. Specify whether the text box should have a border. To add a border, click on the **Border** box, and enter a Thickness value between **.05** and **10**.
2. Define the **Justification** of the text. This justification applies to the position of the text in the box, not the position of the text on the page.
3. Define the Background. Select either **Transparent** or **Opaque**.
4. Click on OK.

Stamp Image

Use the Stamp Image option to select the image that you want to use for the Stamp option. To select the image, choose Stamp Image. The Stamp Image dialog box prompts you to enter a file name and path:

- If you know the name and location of the image file, enter it in the File box.
- If you do not know the name and location, of the image, click on the browse button to search for the file.

After you have selected the image file, click on OK.

Editing Annotation Objects

Once you have annotated an image, you can edit the annotation objects as needed. There are three ways to edit objects:

- **Use the Attributes menu** - The Attributes menu, as noted in the Defining Annotation Attributes section of this chapter, allows you to either set the attributes for adding specific types of objects, or to select existing objects and modify them as needed. Refer to the Defining Annotation Attributes section for more details.
- **Use the Edit menu** - The Edit menu contains general editing features such as Undo, Copy, Cut, Send to Back, etc…
- **Right-click on the report object** - Right clicking on a report object brings up a customized editing menu that contains both general editing commands and the attributes that can be edited for that object.

You can also move and size annotation objects:

- To move an object, left click on it and drag the item to a new location. When you drag the item, the cursor becomes a four pointed arrow.
- To size an object, left click on it and drag the handles to resize the object as needed.

The following sections describe the available editing options.

Undo Undo the last action. Unlike the image capture/editing Undo feature, the annotation Undo undoes up to ten changes.

Bring to Front Bring the selected annotation in front of all other annotations.

Move Forward Bring the selected annotation one layer forward.

Move Backward Send the selected annotation one layer back.

Send to Back Send the selected annotation behind all other annotations.

Cut Remove the selected annotation and place it on the clipboard.

Copy Copy the selected annotation clipboard.

Paste Place the cut or copied annotation from the clipboard into the image.

Clear (Mac) Delete the selected annotation object(s).

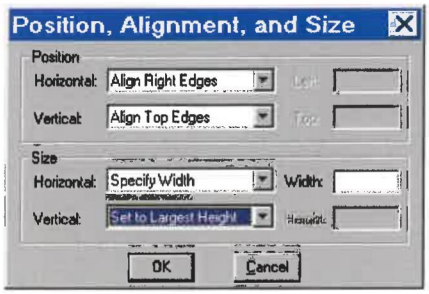
Select All Select all annotation objects.

Duplicate Create additional copies of the annotation object. The program prompts you to enter the number of copies. When you click on OK, the duplicates appear at the top of the window.

Delete (Windows) Delete the selected annotation object(s).

Delete All Delete all of the annotations on the image.

Set Position, Alignment, and Size The **Set Position**, **Alignment**, and **Size** option allows you to specify precise sizing and position values for single or multiple selected report and annotation objects.



The **Position** options enable you to:

- Align top/bottom edges of multiple objects.
- Align left/right edges of multiple objects.
- Align the centers of multiple objects.
- Center an object(s) on the page.
- Enter exact pixel values position the object.

The **Size** options enable you to enter:

- Exact width/height in pixels for one or more objects.
- Set the width/height dimension for multiple objects the to smallest or largest object's in pixels.

Memo

In the SPOT software, you can attach a memo to TIFF or JPEG format image files. To use the Memo option, follow these steps.

1. From the Edit menu, select Memo. The Image Memo window appears for you to enter a new memo or edit an existing memo.
2. Enter the message.
3. Click on OK to attach the memo to the image.

Note: If you attach a memo to an image that is not in either TIFF or JPEG format and then attempt to save the memo (i.e., when you close the image file), the SPOT program displays a warning message that asks you if you want to save the image without the memo. If you want to retain the memo, click on Cancel and use the **Save As** *option to save the image in either TIFF or JPEG format.*

Ch. 9 – Using SPOT Databases

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Introduction

SPOT's image archiving database features are not yet available for the Mac edition of the program. As soon as this functionality is available, you will be able to download the updated version of the software from Diagnostic Instruments' web site, **www.diaginc.com**. Check the site periodically to determine the most recent update. For detailed steps on downloading the software, refer to the **Free Software Updates** section in "About this Manual".

In addition to the numerous image capture and editing features, the SPOT software provides you with an easy to use, fully featured image archiving database.

Note: If you are using SPOT databases from an earlier version of the SPOT software, you will need to convert the old databases to work with the latest version (3.5) of the software. Refer to Appendix E: Converting Previous Version SPOT Databases for detailed steps.

This chapter is divided into the following sections:

- The Database Menu
- The SPOT Sample Database
- Creating and Modifying Databases
- Searching Databases

The Database Menu

The Database menu contains all of the options for accessing, creating, modifying, and searching SPOT databases. When you first open the SPOT program, only the first two options, **New** and **Open** are active. The other options remain inactive (grayed) until you either create a new database or open an existing database. In addition, **Edit Database Record** does not appear on the menu unless a database image is open. Each option is described below.

- **New** – Create a new SPOT database. Refer to the "Creating and Modifying Databases" section for details.
- **Open** – Open or delete an existing SPOT database.

Note: Only one database can be opened per session. If you try to open a second database, the program prompts you to close the open database.

- **Close** – Close a SPOT database.
- **Password** – Define a password to protect a SPOT database.
- **Fields** – Define the text and numeric fields, look-up lists, and default options for your database.
- **Preferences** – Define the location and format of the image files in your database.
- **Search** – Define parameters for database searches.
- **Edit Database Record** - Edit information associated with a database image, including field data, time and date information, file save location, and memo text.

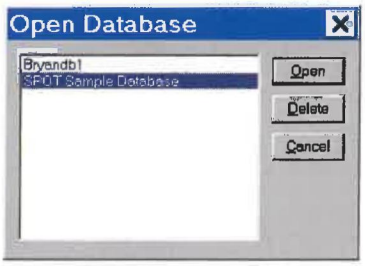
The SPOT Sample Database

For your reference, a fully functional sample database is included with your software. We recommend that you browse through this database to familiarize yourself with the features and functionality. The following section is a "visual tour" of the SPOT Sample Database.

Open the SPOT Sample Database

1. From the Database menu, select **Open.** The SPOT program prompts you to select the database.

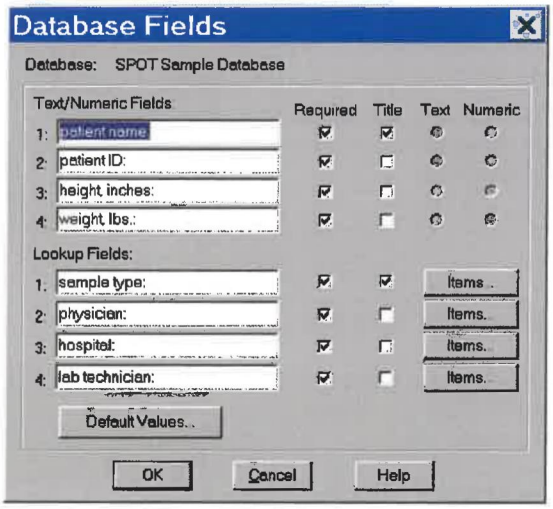
2. Select **SPOT Sample Database**, and click on **Open**. SPOT prompts you for a password.



Note that the SPOT database icon appears at the bottom of your Windows screen, indicating that the database is open.

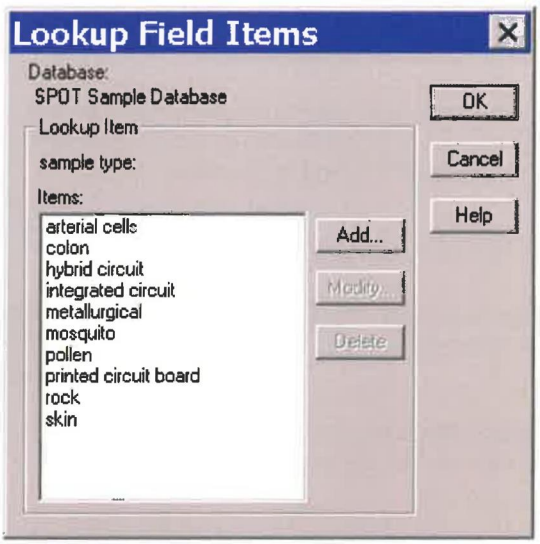
View the Database Structure

From the Database menu, select **Fields**. The **Database Fields** window illustrates the different types of fields that you can define for a SPOT database:



Click on the **Items** button for the **Sample Type** lookup field to view a list of the sample type options for the SPOT Sample Database.

From the **Lookup Field Items** window, you can Add, Modify, or Delete items in the lookup list.



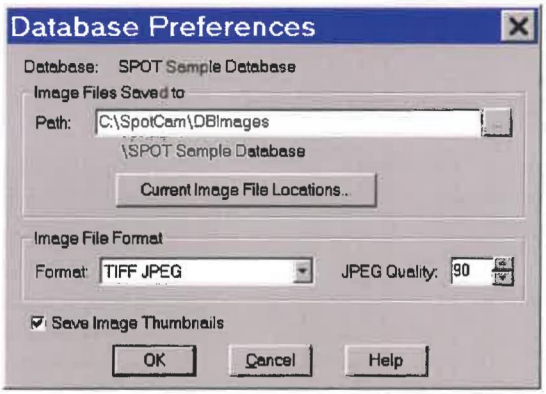
View Database Preferences

From the Database menu, select **Preferences**

The Preferences window is the starting point for defining:

- Image file/database location
- Image file format
- Thumbnail save options

Refer to the **Defining File and Save Preferences** section of this chapter for details.

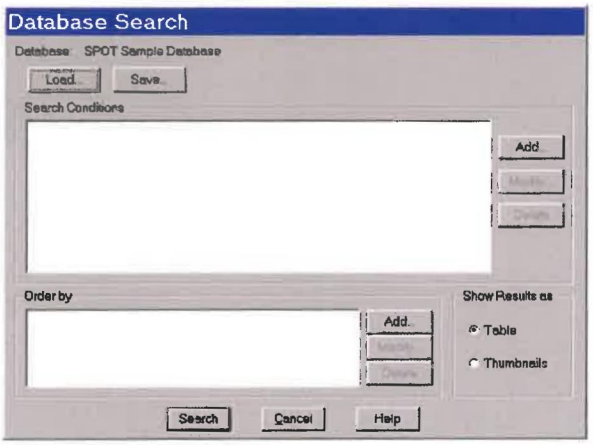


Search the Database

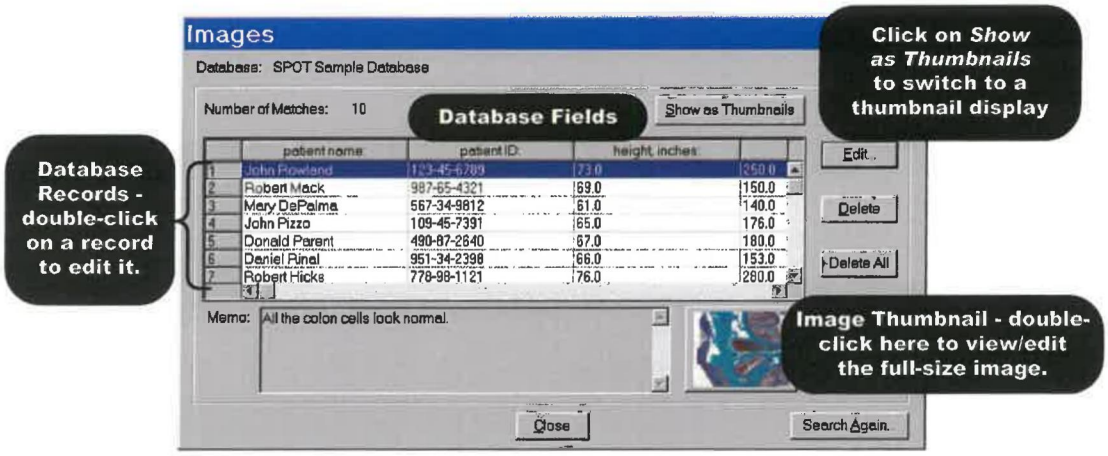
From the Database menu, select **Search.**The Database Search window allows you to:

- Define search and sort parameters
- Save a specific set of search parameters
- Choose display options for search results (i.e., tabular or thumbnail display).

Refer to the **Searching Databases** section of this chapter for details.

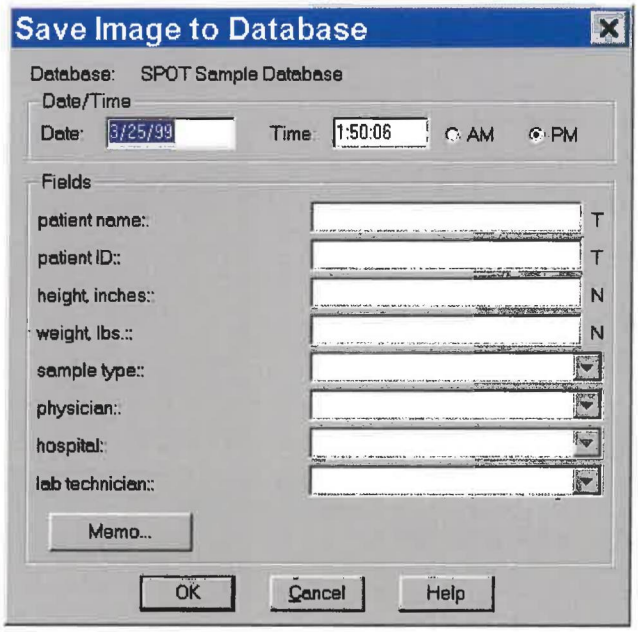


Click on Search. Clicking on Search without entering any conditions results in a display of all the records in the database:



Save an Image to the Database

Open an image file, and from the File menu, select Save to Database:



All of the required fields must be entered before the image can be saved.

Creating and Modifying Databases

The following sections outline the details involved in creating and/or modifying SPOT databases.

- Setting Up the Database
- Defining Database Fields
- Defining File and Save Preferences
- Saving Images
- Editing Database Image Records
- Moving Existing Database Images

Setting Up a New Database

The initial set up for a new database consists of two steps:

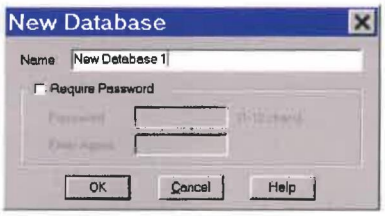
- Naming the database (required)
- Defining a password for the database (optional)

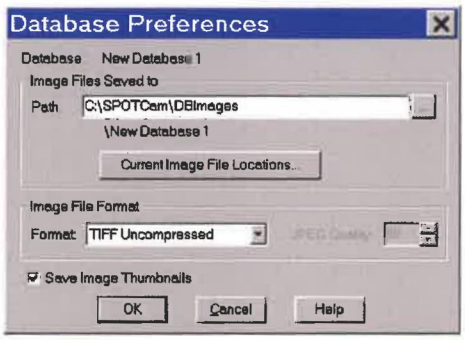
> *Note: If you do not define a password during the initial definition, you can use the Password option on the Database menu to add a password at a later point..*

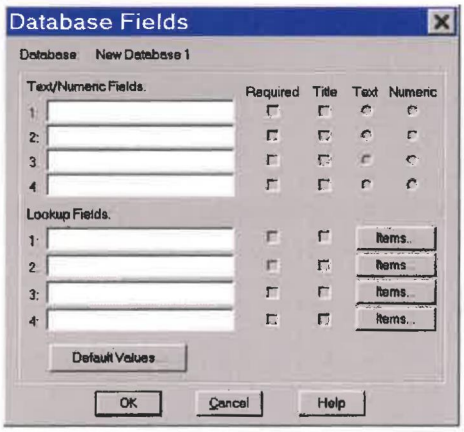
To set up your database, follow these steps:

1. From the Database menu, select **New**.
2. Enter a **Name** for the database.
3. Define a password for the database, if needed. Follow these steps:
   a) Check the Require Password box. The Password and Enter Again boxes are activated.
   b) Enter a 1-10 character alphanumeric password.
   c) Re-enter the password.
4. Click on **OK**. The **Database Preferences** window appears. At this point, you can:
   – Define the database preferences. Refer to the next section, **Defining File and Save Preferences**, for details.
   – Click on **OK to** skip defining **Database Preferences** and proceed directly to defining the database fields and lookup lists (i. e., the **Database Fields** window). Refer to the **Defining Database Fields** section of this chapter for details.

> *Note:* **Database Preferences** *can be defined/modified at any future time by selecting the* **Preferences** *option on the Database menu.*



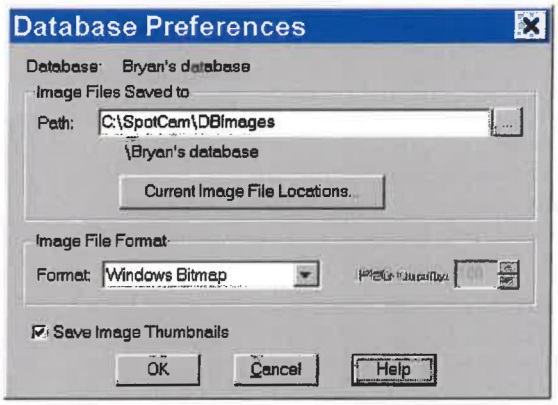




Defining File and Save Preferences

Before saving images to a database, you should specify your file and save preferences for the database. This includes:

- Location and path for database images
- Image file format
- Thumbnail save preference



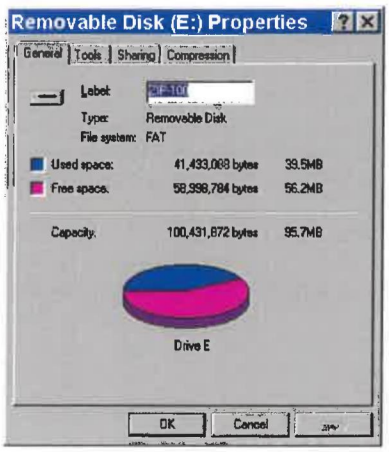
All of the file and save preferences are defined from a single point, the **Database Preferences** window. The Database Preferences window automatically appears when defining a new database. If you want to define the file and save preferences at a later point, select the **Preferences** options from the Database menu.

Storing Archived Images – An Important Consideration

Before proceeding to define the file and save parameters for your database, you should carefully plan how and where you will store archived images, as well as how you will accommodate future images. Because each uncompressed image file ranges from between 1.3 Mb to 8 Mb, saving and storing large quantities of images requires planning. One option for storing images locally is to use the new 2 Gb Jaz drives. Another alternative, if you are on a network, is to designate network space on a server. For more details on storage planning, contact your MIS department or a qualified computer or network specialist.

Location and Path of Database Images

SPOT databases are structured so that the database and the images stored in that database do not have to reside in the same location. This structure allows you to keep the relatively small database program files on your hard drive, while storing the larger image files in a different location. The name and location of the database - **SPOT Sample Database** in the illustration above - appears under the path for the database images; it cannot be changed from this window. The database always resides in the default location, **C:\SpotCam\DBImages**. Although the default location for image files is also **C:\SpotCam\DBImages**, we recommend that you define an alternative location such as a Jaz drive or a network drive. If you plan to save images to removable media such as ZIP or Jaz drives, you should, at this point, define the volume label for the disk. Because a single database of images can span more than one disk, defining unique volume labels for each disk provides a reference so that you can conveniently access images in the future.



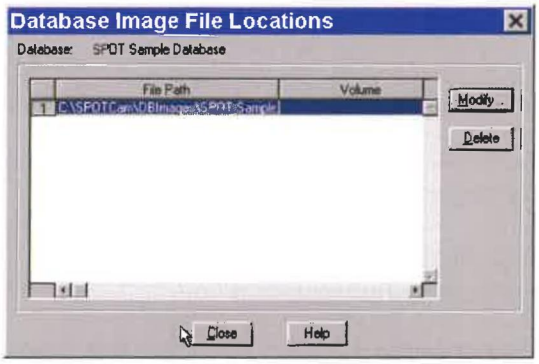
To define a volume label for a disk, follow these steps:

1. Ensure that the ZIP or Jaz disk is in the drive.
2. From the Windows 95/98/NT Explorer, single click on the ZIP or Jaz drive icon.
3. From the File menu, select the **Properties** option; or, right click on the drive and select the Properties option. The Properties window appears. If you do not see a view similar to that in the illustration at right, click on the **General** tab.
4. Enter a name in the **Label** box. For ease of reference, you

should name the volume so that it tells you both the name of the database and the disk number.

5. Click on **OK**.
6. Write the volume label on the ZIP or Jaz disk. This way, when you search a multi-disk image database, and the program prompts you to enter the disk number, you will be able to find the information.
7. Repeat steps 1 – 6 for each disk of images associated with a database.

If you are saving images to an existing database, you can click on the **Current Image File Locations** button to display the location of the saved image files and change the location and/or volume label for the disk. The Database Image File Locations window appears.



To change the File Path and/or the Volume label, either double click on the File Path/Volume information, or select the information and click on Modify. The **Image File Location** dialog box appears.

From this window you can:

- Enter a new location for the database images in the Path box; or, click on the button next to the Path box to browse for a new location.
- Enter a new Volume label for the disk.

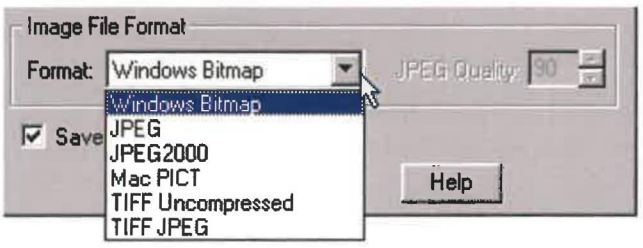
Image File Format

From the Preferences window, you can define the graphic format of the images saved to a database. There are six options:

- Windows Bitmap
- JPEG *(allows for image compression)*
- JPEG2000
- Mac PICT
- TIFF Uncompressed
- TIFF JPEG *(allows for image compression)*

Windows Bitmap is the default option.

To define image file format, select one of the formats from the drop-down list. Selecting either the JPEG or the TIFF JPEG option activates the JPEG Quality box. This option allows you to compress images (i.e., lower their resolution) so that you can save disk space in cases where high resolution images are not needed.



To define JPEG Quality, select a value between 1 and 100:

- A value of 1 represents the lowest resolution and the smallest file size.
- A value of 100 represents the highest resolution and the largest file size.

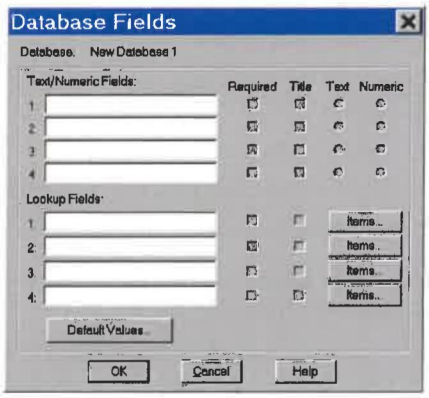
Thumbnail Save Preference

Determine whether you will **Save Image Thumbnails** for the database. The Save Image Thumbnails option saves a thumbnail of each image in the database. This allows you to visually browse through the images in a database. You can also open a database image from a thumbnail.

CAUTION!

When you select the Save Image Thumbnails option, the thumbnail images are saved to the SPOT database file, which resides on your hard drive at C:\Spotcam\Database\[name of your database].db. Each thumbnail increases the size of the database file by approximately 6 Kb.

Defining Database Fields

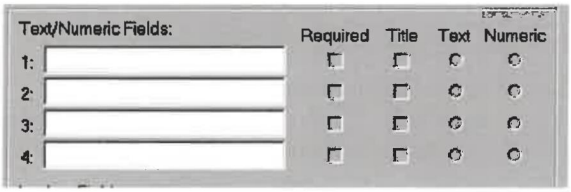


The Database Fields window is a centralized point for defining the details associated with a database. There are three processes associated with the definition of database fields:

- Naming and defining **Text/Numeric Fields** – The textual/numeric fields that you define represent the data you want to be associated with an image. Typical text fields include patient name and ID number. Typical numeric fields include height and weight.
- Naming and defining **Lookup Fields**– Lookup fields also represent data that you want to be associated with an image. Lookup fields are used in cases where the data item has a limited number of discrete options (e.g., blood type). Each lookup field is associated with a user-defined list of items, enabling you to select an option from a list, as opposed to manually entering it each time an image is saved to the database.
- Setting **Default Values** for the fields – To speed the process of saving images to a database, you can define default values to automatically appear for a field. This helps you to avoid multiple entries of the same item when numerous images have the same data for a particular field.

Text/Numeric Fields

To define a textual/numeric field, follow these steps.



1. On the Database Fields window, enter the name of the field in the Text/Numeric Fields box. You can use up to 30 alphanumeric characters. Entering information in the box activates (un-grays) the four variables to the right of the box.
2. Define the four variables associated with each field.
   - **Required** – Check (click on) the Required box to make the field a required entry when saving images to the database. When this box is checked, images can not be saved until the field has an entry.
   - **Title** – Check the Title box to ensure that the data from the text field appears in the title of the image window and under any thumbnail of the image.
   - **Text** – Click on the Text button to define the field as a text field. This is the default setting for the field type.
   - **Numeric** – Click on the Numeric button to define the field as a numeric field.

The field type (i.e., text or numeric) is important in terms of how the program searches the database. Text field searches look for textual strings of information while numeric field searches look for a range of values. So, a database field that contains numbers should not automatically be defined as a numeric field. For instance, if your field is a number such as a patient ID, it should be defined as a Text field because it is not a measurement and it is not within a specific range of values.

CAUTION!

After you have defined the Text/Numeric fields, you cannot change the Text or Numeric setting. Although you can make changes to an existing field name (i.e., to correct a spelling error or make a more intuitive name), you should not change the meaning of the field. This will render the field useless for searches performed on all previously stored images.

Lookup Fields and Lists

Defining a Lookup Field consists of three actions:

- Naming the Lookup Field
- Selecting the Required and Title variables
- Creating the items in the Lookup Field list

The SPOT database allows you to perform these actions in different orders, depending on your situation. Consider the following scenarios:

- You are the database administrator, and you want to define a new database with multiple Lookup Fields for others to use when saving images to the database. You know ahead of time which Lookup Fields you want to define, and you also know all of the items that will be associated with each Lookup Field.

  In this scenario, you can define the Lookup Fields, the Required and Title options, and some or all of the items in Lookup Field list.

- You are an end user and know which Lookup Fields you want to define, but prefer to add items for each field, as needed, when saving images to the database.

  You can define one or more Lookup Fields without adding any of the Lookup Field list items. The Lookup Field items can be added either at a later time, or during the process of saving images to the database.

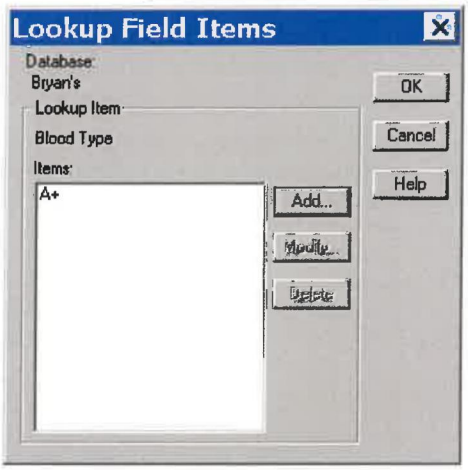
To define a Lookup Field and the associated list, follow these steps:

1. Enter the name of the first Lookup Field.
2. Select the **Required** and **Title** options.
   - **Required** – Check (click on) the Required box to make the field a required entry when saving images to the database. When this box is checked, images can not be saved until the field has an entry.
   - **Title** – Check the Title box to ensure that the data from the text field appears in the title of the image window and under any thumbnail of the image.

   You can also leave one or both options unchecked. If you do not want to enter the Lookup Field list items at this time, you can enter any remaining Lookup Fields and click on **OK** to save the database.
3. Click on the **Items** button. The **Lookup Field Items** window appears with the name, as shown at right.

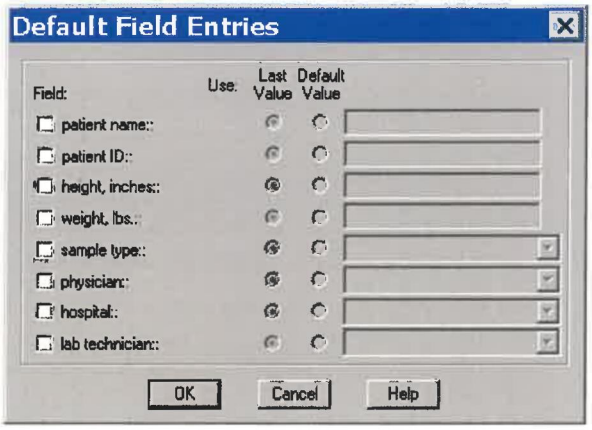
4. Click on **Add**. The **Lookup Item** dialog window appears for you to enter the list item.
5. Enter the name of the list item in the box.
6. Click on OK. The program returns you to the Lookup Field Items window. In the illustration to the right, the user has added the **A +** item to the Blood Type Lookup Field.

   From the Lookup Field Items window, you can:
   - Click on **Add** to add additional items to the Blood Type lookup list.
   - Select a lookup list item and click on **Modify** to edit the item or **Delete** to remove the item from the list.
   - Click on **OK** to save the lookup list and the associated items. The program returns you to the Database Fields window.
7. Follow steps 1-7 to enter additional Lookup Fields. You can enter up to four per database.



Setting Default Values

As part of the process of defining database fields, you have the option to define default values for each field. The values that you define will appear as default field entries each time you save an image to the database. If desired, you can edit the default entries before saving the image. To enter default values for a field, follow these steps:



1. After you have defined your text/numeric and lookup fields, click on **Default Values** (at the bottom of the Database Fields window). **The Default Field Entries** window appears, as shown at right. Unless specified, the SPOT database uses the Last Value for each field. To change this default for a Field, check the box next to the field name. Checking the box activates the Use: options.
2. Select the option that you want:
   - **Last Value**
     When saving an image to a database, the program uses the data entered for the last image as the entry for the same field in the current image. The last entered data always appears in the entry box for the image that you are saving. Thus, if you select Use: Last for the physician field, and you select Dr. Jarvis for the first image, Dr. Jarvis appears in all subsequent images until you make a different selection.

     To select the Use: Last option, click on the Last button for that field and click on OK to save the settings.
   - **Default**
     When saving an image to a database the program uses the entry that you make (in the box next to the Default button). If desired, you can edit the default entries before saving the image.

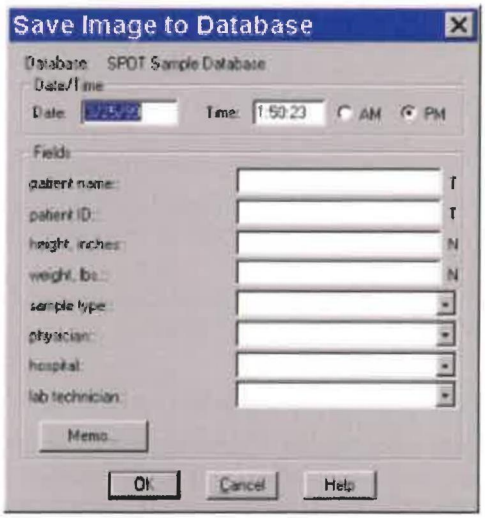
     To select the Use: Default option, click on the Default button for the field and enter/select the default entry. Click on OK to save the settings.

If you do not specify a default value option for a field, all of the fields must be entered/selected each time an image is saved to the database.

Saving Images

Once you have acquired the images that you want to store in your database, the SPOT program makes it easy to save the information. To save an image to a database, follow these steps:

1. From the File menu, select the Save to Database option. The Save Image to Database window appears. The program automatically displays the date and time that you first saved the image. This information can be modified as needed.



> *Note: When you save an image to a SPOT database, the program automatically creates an image ID that is stored in the image path folder. This is a reference number that the program uses to access images and associated data.*

2. Enter the information associated with the image in the database fields.
   - **T** next to a field indicates that the field is Textual.
   - **N** next to a field indicates that the field is Numeric.

   To enter data for a lookup field that is not contained in the drop-down list options, simply type the information into the field.

> *Note: If you defined a data field as* **Required** *on the Database Fields window, the program will not let you save the image without the information.*

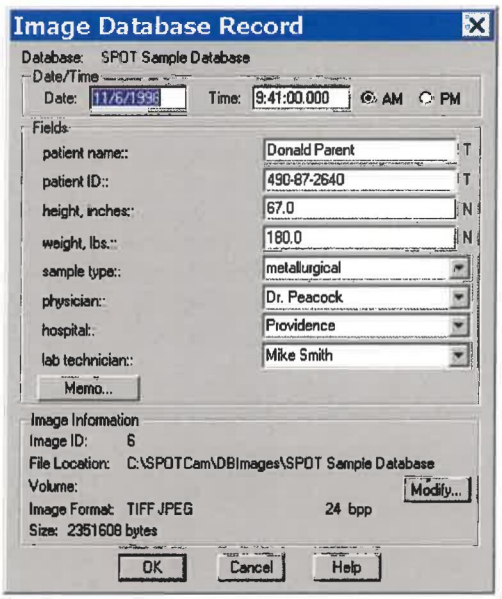
3. Click on the Memo button to associate a memo with an image. The Image Memo window appears.
4. Enter the text for the memo, and click on **OK** to save the memo.
5. Click on **OK** at the Save Image to Database window. If you entered a lookup list item that was not in the drop-down list of options, as described in step 2, the program prompts you to add the item to the list.
6. Click on Yes to add the new item to the lookup list.

Editing Database Image Records

Once you have created a database and saved images to that database, you can edit records as needed. To edit a database image record, follow these steps:

1. Ensure that the database is open.
2. From the File menu, select **Open Database Image**. The dialog box prompts you to enter the Image ID. If you do not know the Image ID, you can search the database, as described in the Searching the Database section that follows.

3. Click on OK to open the database image.
4. From the Database menu, select the Edit Database Record option. **The Image Database Record** window appears.
5. Change the Date/Time settings as needed. Use the following format:
   mm/dd/yy.
6. Edit the Fields data as needed.
   - For the text and numeric fields, simply enter the new data.
   - For the lookup fields, either select an item from the drop-down list or enter a new item. When you close the Image Database window, the program prompts you to add the new lookup list item.
7. Edit the Image Memo, if necessary. Click on **Memo**, enter the information, and click on OK.



In addition to the data associated with a database image, the Image Database Record window also allows you to modify the image file location/path information. For example, if you have moved a database image to a new file location, you would need to modify the record to reflect this.

8. To modify the location/path information for a database image, click on Modify. The Image File Location window appears.

9. Either enter the new file path in the Path box, or click on the button to browse for the information.
10. Enter the new Volume label.
11. Click on OK.
12. Click on OK at the Image Database Record window to save the changes.

Note: The image itself cannot be edited while the Image Database Record window is open.

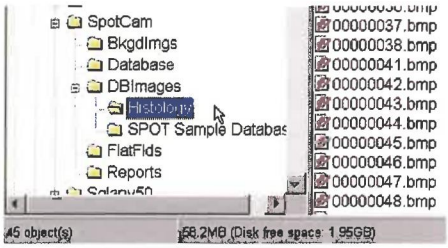
Moving Database Images

When saving images to a database, the default save location is your computer's hard drive – **C:\SpotCam\DBImages** (or wherever you installed the SPOT software). To move images in a database from your hard drive to a removable storage medium such as a zip disk or a CD, follow these steps.

1. **Determine the file space needed for the database images.**

   a) Open the Windows Explorer.

   b) Navigate to the database image files. In the example at right, you can see that the image files associated with the **Histology** SPOT database are located in the **C:\SPOTCam\DBImages\ Histology** directory. Note that the status bar at the bottom of the screen displays the number of objects in the **Histology** folder (45) and the disk space used by these objects (58.2 MB).

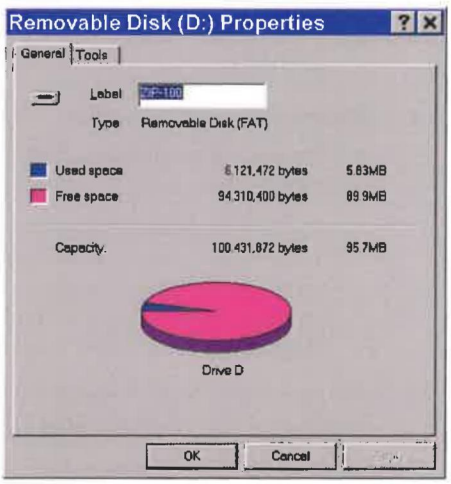


2. **Define the volume label for your zip disk.**
   In some cases, you will be able to store all of a database's images on one disk. At a future point, you might want to add additional images to the database that will not fit on the same disk. Defining a volume label enables you to access SPOT databases that span more than one Zip disk (or any other removable media). To define a volume label, follow these steps:

   a) Insert the Zip disk (or other removable media) in the drive.

   b) In Windows Explorer, right click on the drive letter for the Zip disk.

   c) Select the Properties option. You should see a view similar to that shown at right. If you do not see the pie shape, click on the **General** tab.



   d) Enter a name for the Volume Label in the **Label** box. You should use a name that indicates both the name of the database and the disk number (e.g., HISTO1).

   e) Click **OK**.

   f) For future reference, label the disk or CD.

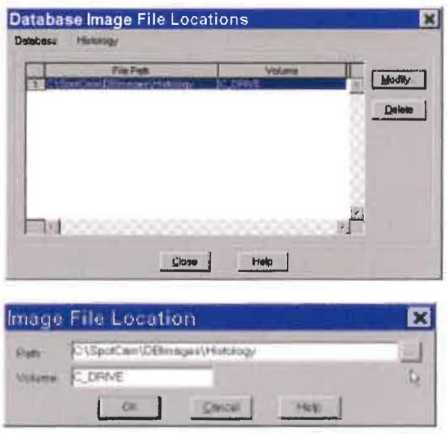
3. **Move the database images to the Zip disk.**

   a) If it is not already open, open the SPOT program.

   b) Open the database that contains the images that you want to move. From the Database menu, select **Preferences**.

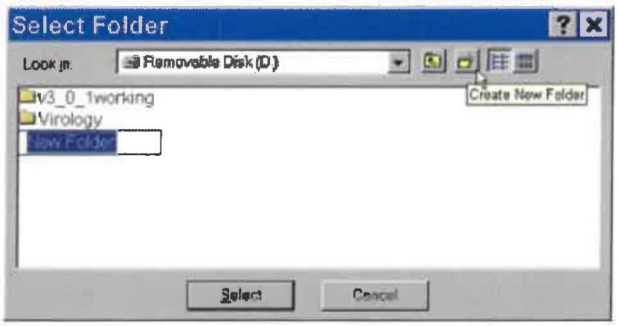
   c) At the Database Preferences window, click on **Current Image File Locations**. The Database Image File Locations window displays the current file path and volume, as shown at right.

   d) Click on the **Modify** button. The Image File Location window appears, with the current path and volume information for the database images, **c:\SpotCam\DBImages\Histology**.



e) Either click on the square box next to the file path to navigate to the folder that you want to move the images to, or enter the file path in the box. When you click on the square box next to the Path box, the SPOT program prompts you with an Explorer view. Navigate to the **D:** drive (or whichever letter is used by your PC).

f) Click on the Create New Folder tab, as shown at right.



Note: If you simply enter the Path **D:\Histology** *on the Image File Location dialog (above), the SPOT program will not create the folder. You must create the folder manually, either from the Windows Explorer, or from the Select Folder dialog.*

g) Enter the name of the new folder, e.g., **Histology**.

h) Click on **Select**. The Image File Location dialog reappears.

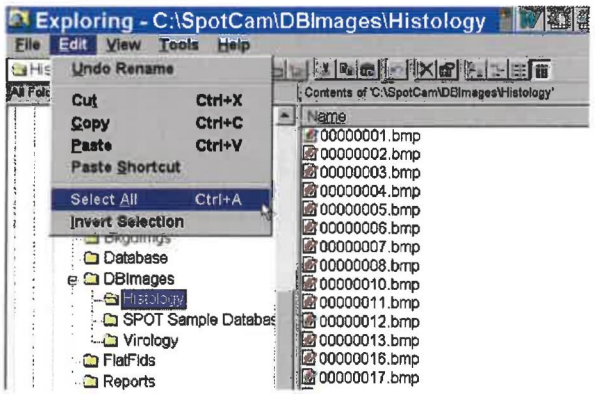
i) Enter the **Volume** label that you defined for the Zip disk in step 2, e.g., HISTO1

j) Click on **OK**. The Database Image File Locations window reappears.

k) **Close** the Database Image File Locations window. The Database Preference window reappears. Note that the **Image Files Saved To** path has not changed – it is still C:\SpotCam\DBImages\Histology. Unless you change this setting, new images added to the database will still be saved to this location.

l) Click on **OK**.

4. From the Windows Explorer, cut and paste the image(s) from the original location (e.g., C:\SpotCam\DBImages\Histology) to the new location (e.g., D:\Histology).



   a) Navigate to the C:\SpotCam\DBImages\Histology folder, and click on Histology.

   b) From the **Edit** menu, choose **Select All**.

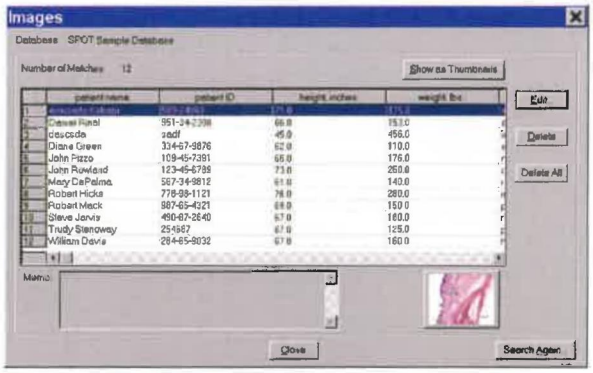
   c) Cut the images **[Ctrl X]**.

   d) Click on the D:\Histology folder.

   e) Paste the images **[Ctrl V]**.

Steps one through four describe how to move an entire database of images. However, it is possible to move individual images or groups of images. When moving individual or groups of images from a SPOT database, you must change the path of *each* image file that you want to move.

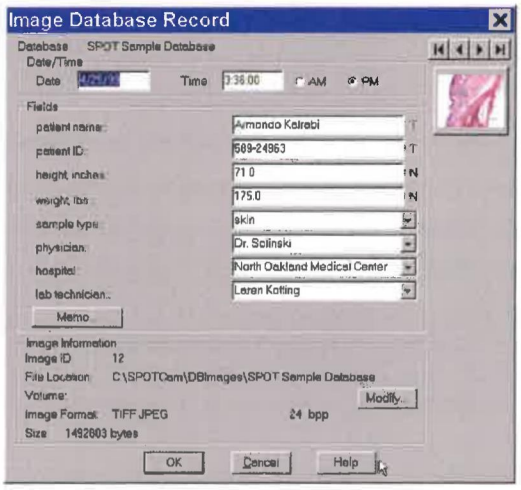
To do so, follow these steps:



1. Search the database to find the image(s) that you want to move. For details on entering search conditions, refer to the Searching Databases section below.

2. From the search results display table (the Images window), select an image that you want to move, and click on Edit. You can select only one image at a time for editing. The Image Database Record window appears.

> *Note: You can also access the Image Database Record window by opening a database image (i.e., **File>Open Database Image**) and then selecting the* **Edit Database Record** *option from the Database menu.*



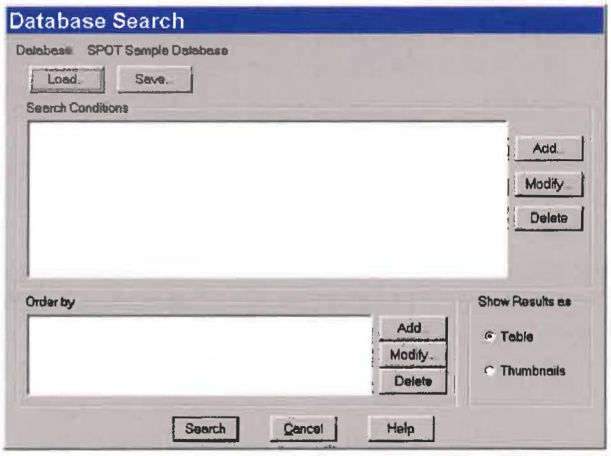
3. Note down the Image ID number – the first item in the Image Information section.
4. Click on the Modify button. The Image File Location dialog appears.
5. Enter the Path and Volume information as described in steps 2d – 2i above.
6. Repeat steps two through five for each image that you want to move.
7. From the Windows Explorer, cut and paste the images from their original location to the new location defined in step five.

Searching Databases

The SPOT program offers a robust, yet easy to use database search function. The **Database Search** window is the central point from which search and display parameters are entered. From this window, you:

- Define search conditions
- Order search results
- Display and edit search results
- Save search criteria
- Load previously saved search criteria

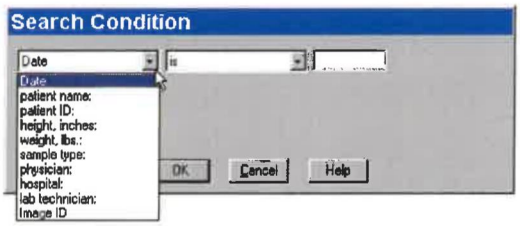
To access the Database Search window, select Search from the Database menu.



Defining Search Conditions

To define database search conditions, follow these steps.

1. Click on the Add button (for Search Conditions) on the Database Search window. The Search Condition window appears.
2. Select the database field that you want to base the search on. The left drop-down box contains a list of all the textual/numeric and lookup fields that you defined for the database. In addition to the fields that you defined, the Date associated with the image and the Image ID appear in the list.



3. Select the criteria for the search from the center drop-down box. The list options vary according to the type of field that you selected, as shown below:

Field Type	Options	Field Type	Options
Date	is is is not is before is on or before is after is on or after is between is not between is one of is not one of	**Numeric**	is equal to is equal to is not equal to is less than is less than or equal to is greater than is greater than or equal to is between is not between is one of is not one of
Text	is is begins with doesn't begin with contains doesn't contain is like (use * and ?) is not like (use * and ?) is one of is not one of	**Lookup**	is is is not is one of is not one of

4. Enter the final search parameter in the right hand box. This entry will vary according to the search criteria that you selected. For **Text** and **Numeric** field searches, you are required to enter the information, while for **Lookup** field searches, you can select from a list of the lookup items

   For Text fields, asterisks (*) and question marks (?) can be used as "wild card" characters with the **is like** and **is not like** options.

   – * - The asterisk wild card tells the program that any number of characters can be used with this text field.

   For example, if you want to search all patients with "smith" in their name, you can enter ***smith***. This search will bring up names such as John Smith, Jane Smithfield, and Joe Hammersmith.

   – **?** - The question mark wild card tells the program that any single character can be used where the question mark appears.

   For example, if you want to search for patients with either the name Smith or Smyth, you can enter * **sm?th**. The asterisk and space in the front of sm?th tells the program to find all first names associated with sm?th. The question mark tells the program to search for one letter difference variations on the name Smith. This search will bring up names such as John Smith, Anne Smyth, and Fred Smoth, but it will not bring up Jane Smithfield, Harry Hammersmith, or Abigail Smythe.

5. Click on OK to save the search parameters. The Database Search window reappears with the search conditions in the box.

   After you have entered a search condition, you can:
   - Click on **Add** to add another search condition.
   - Select a condition and click on **Modify** to change it.
   - Select a condition and click on **Delete** to remove it from the search.

6. Define how the search results will be ordered. Refer to the next section for details.

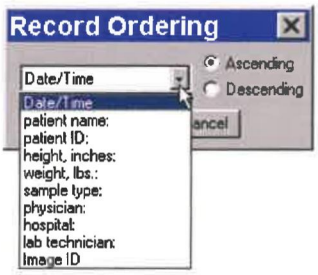
Ordering Search Results

After you have defined the search conditions, the **Order by** options allow you to set the display order of the search results.

To order search results, follow these steps:

1. Click on the Add button to the right of the Order by box. The Record Ordering window appears.
2. Select from the list of drop-down list options to determine how the database search results will be ordered. In addition to the database fields that you defined, the options include Date/Time and Image ID.
3. Check either the Ascending or Descending option:
   - Ascending lists display results beginning with the lowest value and progressing to the highest value.
   - Descending lists display results beginning with the highest value and descending to the lowest value.
4. Click on OK. The options that you selected appear in the Order by box:

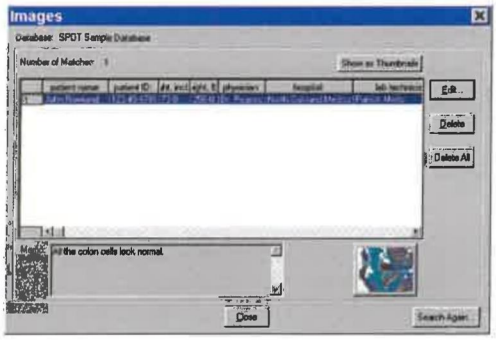
   After you have entered an ordering condition, you can:
   - Click on **Add** to add another ordering condition.
   - Select a condition and click on **Modify** to change it.
   - Select a condition and click on **Delete** to remove it from the search.
5. Choose the display options. Refer to the next section for details.



Displaying Search Results

The SPOT software allows you to display database searches either in tabular form or as thumbnails. From the **Show Results as** box, select either Table or Thumbnails. When all the search conditions have been entered, click on Search. The following sections detail the display and editing functionality.

Show Results as: Table



Display the search results in a table. From left to right, the column headings for the table are:

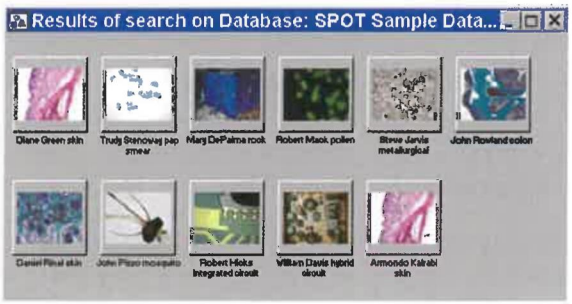
- The user-defined database fields
- Date/Time
- Image ID
- Image Format
- Bits per Pixel
- Size (in bytes)

The illustration at right shows a search that resulted in one record.

From the table window, you can:

- **Open a database image** – Select the database record item, and click on the thumbnail at the bottom of the window. The image opens for viewing and modification.
- **Edit a database record** – Either select the database record item and click on Edit, or double-click on the database record item. The Image Database Record window appears. Refer to the Editing Database Image Records section below for details.
- **Delete a single database record** – Select the database record item and click on Delete.
- **Delete all of the database records** - Select a database record item and click on Delete All.
- **Switch to the thumbnail view** – Click on the Show as Thumbnails button.
- **Perform another search** – Click on the Search Again button to return to the Database Search window, where you can add or modify search conditions.

Show Results as: Thumbnails



Display the search results in the form of image thumbnails. The illustration to the right shows the thumbnail view of a database search that produced eleven records.

- ***Double-click*** on a thumbnail to open an image for viewing or modification.
- ***Right-click*** **(Windows)/[Ctr] Click (Mac)** on a thumbnail to display a list of options, as shown at right. The right-click menu enables you to perform a variety of actions directly from the thumbnail window. You can:
  - **Select** an image.
  - **Open** a database image.
  - **Delete** an image from the database.
  - **Print** an image.
  - **Edit Database Record** -Edit the database image record.
  - **Add Image to Image Sequence** – Add a database image to an image sequence or create a new image sequence.
  - **Add Image to Report Template** – Add a database image to a selected report template. When you select this option, SPOT prompts you to select from a list of reports. If you have not defined a report, SPOT automatically creates a new report for you and inserts the image as a **Static Image** object. Refer to the **Creating Reports** section of Ch. 10 - Reporting for more details.
  - **Select all images** in the thumbnail view. When you use this option, the right-click menu expands to include the above options for multiple images.
- ***Click and drag with the mouse*** to select two or more images.

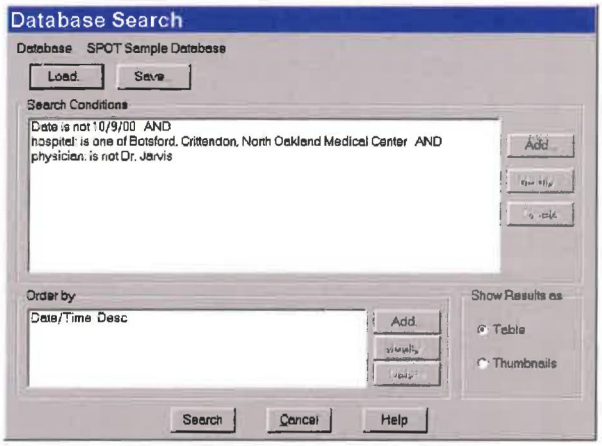
Saving and Reusing Search Criteria

After you have defined the search and display parameters, you can save that set of criteria to be used for future searches on that database. Once saved, you can then reuse the search criteria clicking on the Load button.

To save a search, follow these steps:

1. Enter the search and ordering parameters.
2. Click on **Save**. The program prompts you to name the database query.
3. Name the query.
4. Click on **OK**. At this point, you can:
   - Run the search by clicking on **Search**.
   - Close the **Database Search** window and reopen the search at a future time.

   To reopen the search at a later time, click on the **Load** button, and select a search (**.qry**) file. Click on **Open**. The Database Search window displays the parameters for that search query.



Editing Database Image Records (from a search)

The SPOT program provides two ways to access and edit database image records:

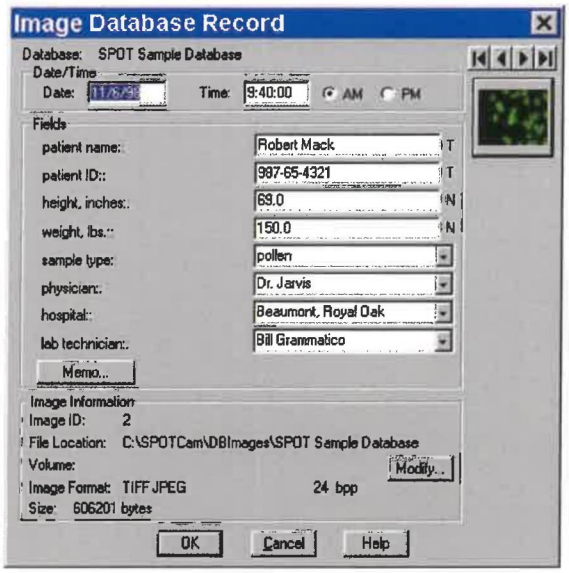
- Via the Edit Database Record option on the Database menu
- Via the display windows of database search results

In both cases, the Image Database Record window allows you to change the information associated with the database image record.

However, the functionality differs slightly, depending on how the window is accessed:

Accessed via the Database Menu	Accessed via a Search Results Display
Modify date/time, database field entries, image memo, and file/path information.	
Modify the database record for the open image only.	Modify the database record for any image in the database.
Edit the database record only.	Edit the database record and/or the image itself.

To edit a database image record from a search results display window, follow these steps:



1. Open the Image Database Record window.
   - If your results are displayed in a table, either double-click on the record in the table, or select the record and click on Edit.
   - If your results are displayed as thumbnails, right-click on the thumbnail.

   The Image Database Record window appears. Notice that navigational arrows appear above the thumbnail.
2. Change the Date/Time settings as needed. Enter the information in **mm/dd/yy** format
3. Edit the Fields data as needed.
   - For the text and numeric fields, simply enter the new data.
   - For the lookup fields, either select an item from the drop-down list or enter a new item. When you close the Image Database window, the program prompts you to add the new lookup list item.
4. Edit the Image Memo, if necessary. Click on the Memo button, enter the information, and click on OK.

In addition to the data associated with a database image, the Image Database Record window also allows you to modify the image file location/path information. For example, if you have moved a database image to a new file location, you would need to modify the record to reflect this.

5. To modify the location/path information for a database image, click on Modify. The Image File Location window appears.
6. Either enter the new file path in the Path box, or click on the button to browse for the information.
7. Enter the new Volume label.
8. Click on OK.

At this point, you can either save the changes to the image record or proceed to make changes to another database record.

- To save the changes and return to the search results display, click on OK.
- To edit another database record without closing the Image Database Record window, use the navigational arrows at the top of the window to browse for the record.
  - Use the two middle arrows to scroll one image at a time through the database.
  - Use the left arrow to go back to the first image in the database.
  - Use the right arrow to go forward to the last image in the database.

9. Repeat steps one through seven to modify additional image database records.

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Ch. 10 - Reporting

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Introduction

The SPOT software includes a built-in reporting functionality that can be used either by itself or in conjunction with a SPOT database (Windows) to collect, organize, and present information related to your image captures. This chapter discusses the process of creating and running reports. It is divided into the following sections:

- Overview: The SPOT Sample Report (Windows)
- Creating a Report
- Running a Report
- Distributing Reports

Overview: The SPOT Sample Report (Windows)

When you create a report in SPOT, you are building a template to be applied to specific situations. Running the report applies the template to an image or images. For example, you might have one template called Histology Report to document histology samples and another template called Cell Pathology Report to document different cell cultures. Similarly, you might want to produce reports for production line analysis. In this case, you could define different templates for different parts, such as Part A Analysis Report, Part B Analysis Report, etc…

For your reference, a fully functional sample report is included with the SPOT software. This report can be used:

- As an introduction to SPOT's reporting features
- As a basis from which to build additional reports

The following section is a "visual tour" of the SPOT Sample Report.

Open the SPOT Sample Report

The SPOT Sample Report, **Pathology Report**, is a database report: it is based on the information associated with the images in the SPOT Sample Database. To open the SPOT Sample Report, follow these steps:

1. Because the SPOT Sample Report is based on the SPOT Sample Database, you must first open this database:
    a. From the Database menu, select Open.
    b. Select SPOT Sample Database.
    c. Click on Open.

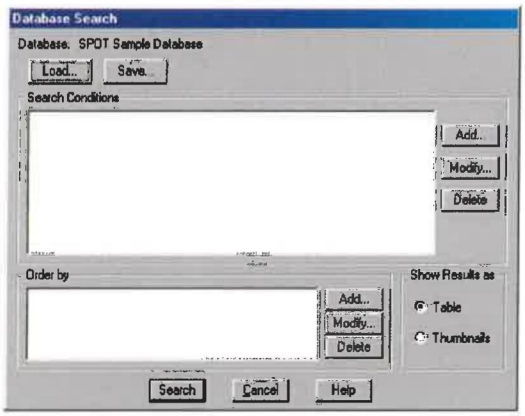
2. Open an image from the SPOT database.

   a. From the Database menu, select Search. The Database Search window appears. Do not enter any search conditions at this point.

   b. In the Show Results as box, select **Thumbnails**.

   c. Click on Search. The search results display.

   d. Click on the **John Rowland colon** thumbnail to open a database image to report on.

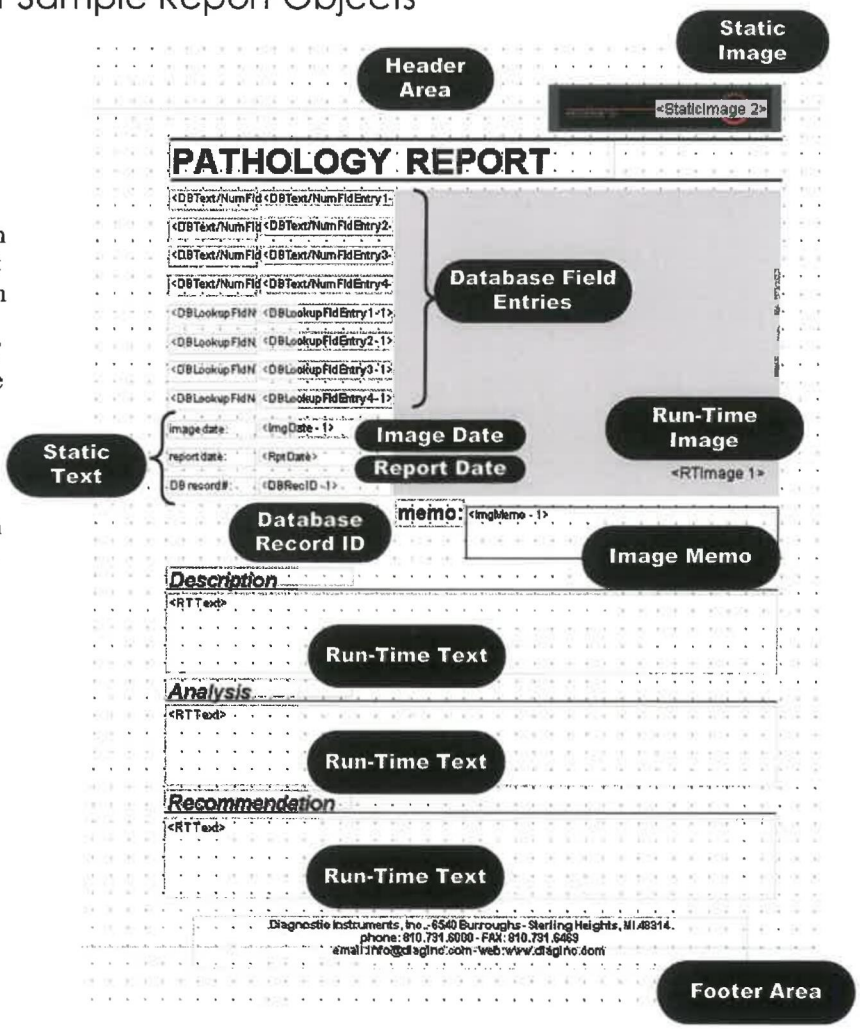


3. Open the SPOT Sample Report. From the File menu, select Open Report. The Open window appears for you to select the file. The file path and name for the SPOT sample report is **C:\Spotcam\sampleDBreport.rpt**.

The report appears in a new window and the menus and menu options in the main window change to reflect SPOT's report editing functions.

View the SPOT Sample Report Objects

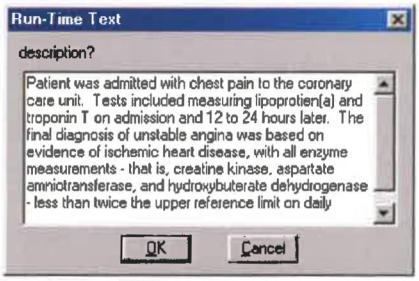
When you open the SPOT Sample Report (or any SPOT **rpt** file), you are actually opening a template of user-defined report objects that the program uses to create the report output. The call outs on the following illustration of the SPOT Sample Report template identify the different objects used in the design of this report. Refer to the **Defining Report Objects** section for descriptions of each object.



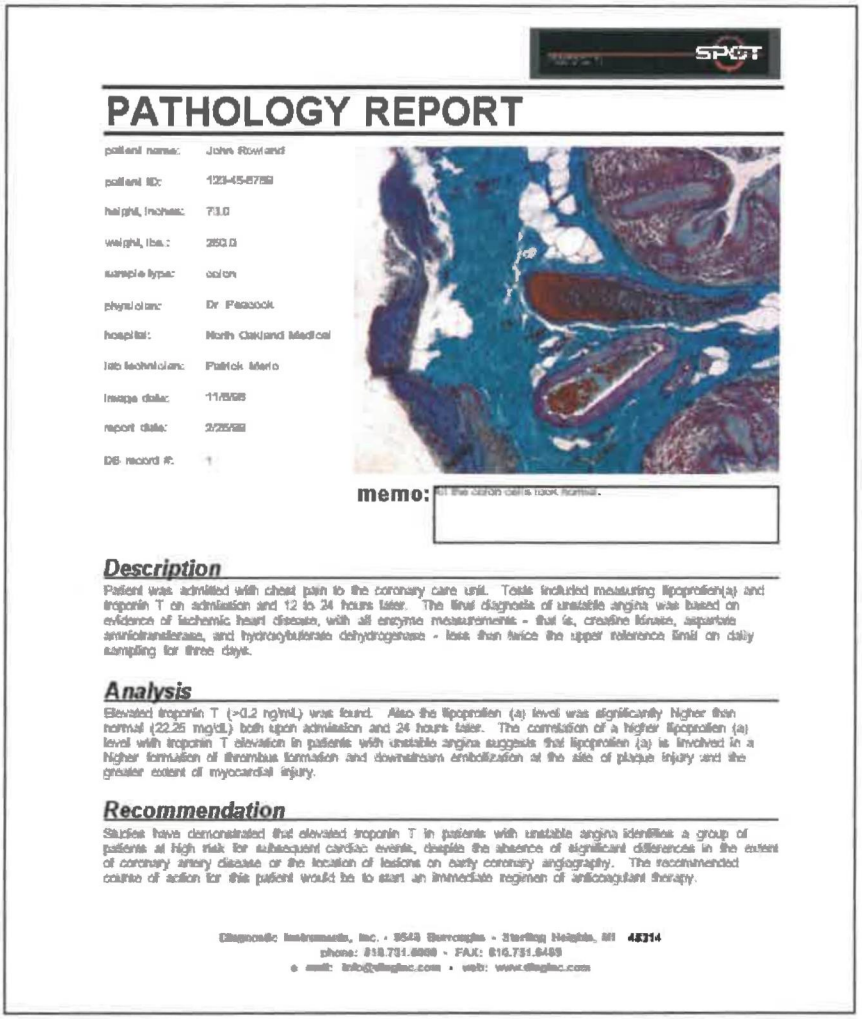
Run the SPOT Sample Report

To run the Pathology Report for the **John Rowland – colon** image from the SPOT Sample Database, select **Run Report** from the File menu. The report sequence is as follows:

1. The Run Report window prompts you to define the Output destination for the report. Select **Preview Window**.
2. Click on **Run**.
3. The report then prompts you with the run-time text prompts that have been defined for this report. Because there are three run-time text prompts for the SPOT Sample Report (i.e., to enter the information for the Description, Analysis, and Recommendation fields), you will be prompted to enter run-time text for each field. In the illustration at right, the run-time text has already been entered, but if you were reporting on the image for the first time, you would see only the prompt. Click on OK at each window. When you click on OK at the third Run-Time text window, the report appears, as shown below.


Run-Time Text
description?
Patient was admitted with chest pain to the coronary care unit. Tests included measuring lipoprotien(a) and troponin T on admission and 12 to 24 hours later. The final diagnosis of unstable angina was based on evidence of ischemic heart disease, with all enzyme measurements - that is, creatine kinase, aspartate amniotransferase, and hydroxybuterate dehydrogenase - less than twice the upper reference limit on daily
OK
Cancel

4. Click on Close to close the report without saving or printing it. Refer to the Running Reports section for more details.



PATHOLOGY REPORT

patient name: John Rowland
patient ID: 123-45-6789
height, inches: 73.0
weight, lbs.: 250.0
sample type: colon
physician: Dr Peacock
hospital: North Oakland Medical
lab technician: Patrick Mario
image date: 11/5/98
report date: 2/25/99
DB record #: 1

memo: of the colon cells look normal.

Description

Patient was admitted with chest pain to the coronary care unit. Tests included measuring lipoprotien(a) and troponin T on admission and 12 to 24 hours later. The final diagnosis of unstable angina was based on evidence of ischemic heart disease, with all enzyme measurements - that is, creatine kinase, aspartate amniotransferase, and hydroxybuterate dehydrogenase - less than twice the upper reference limit on daily sampling for three days.

Analysis

Elevated troponin T (>0.2 ng/mL) was found. Also the lipoprotien (a) level was significantly higher than normal (22.25 mg/dL) both upon admission and 24 hours later. The correlation of a higher lipoprotien (a) level with troponin T elevation in patients with unstable angina suggests that lipoprotien (a) is involved in a higher formation of thrombus formation and downstream embolization at the site of plaque injury and the greater extent of myocardial injury.

Recommendation

Studies have demonstrated that elevated troponin T in patients with unstable angina identifies a group of patients at high risk for subsequent cardiac events, despite the absence of significant differences in the extent of coronary artery disease or the location of lesions on early coronary angiography. The recommended course of action for this patient would be to start an immediate regimen of anticoagulant therapy.

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Creating a Report

To create a new SPOT report, select **New Report** from the File menu. The following changes take place in the main window:

- The report definition window appears as a separate window. If an image or image(s) were open, they remain open.
- The **Object** menu appears. Use these options to specify the fields, text, and images that appear in the report.
- The **Attributes** menu appears. Use these options to define the appearance and style of the report.
- The **View** menu options change to reflect report definition function.
- The **Edit** menu options change to reflect report editing functions. These options are available only after one or more report objects has been added to the New Report window.

Note: When you move between an image window and the report definition window, the menu options change accordingly.

This section is divided as follows:

- **Working in Report Mode**– general reporting features and functionality
- **Defining View Options**– the view of the report definition screen
- **Defining Report Attributes** – the style and appearance of the report
- **Defining Report Objects** – the text, field, and image information that appears in the report
- **Editing Report Objects** – edit options for the report objects

Working in Report Mode

The report mode contains functionality that differs from the image capture and editing functionality. The following is a brief overview of these features.

Cursor Functionality

Cursor functionality in SPOT reports varies according to which **Object** menu option is selected.

- If **None** is selected, the cursor is a an editing tool for selecting, sizing, and positioning objects. When creating new reports, **None** is the default option.
- If a report object is selected, the cursor changes to a cross-hair icon for placing new report objects to the grid.

Sizing and Positioning Report Objects

When sizing and positioning a report object in the report window, you must first select the object by single clicking on it. When an object has been selected, handles appear at the corners; for line objects, the handles appear at each end. You can select multiple report objects by holding the mouse button down and dragging the cursor around the objects. Handles appear around both objects. When selected, report objects can be sized or positioned as needed.

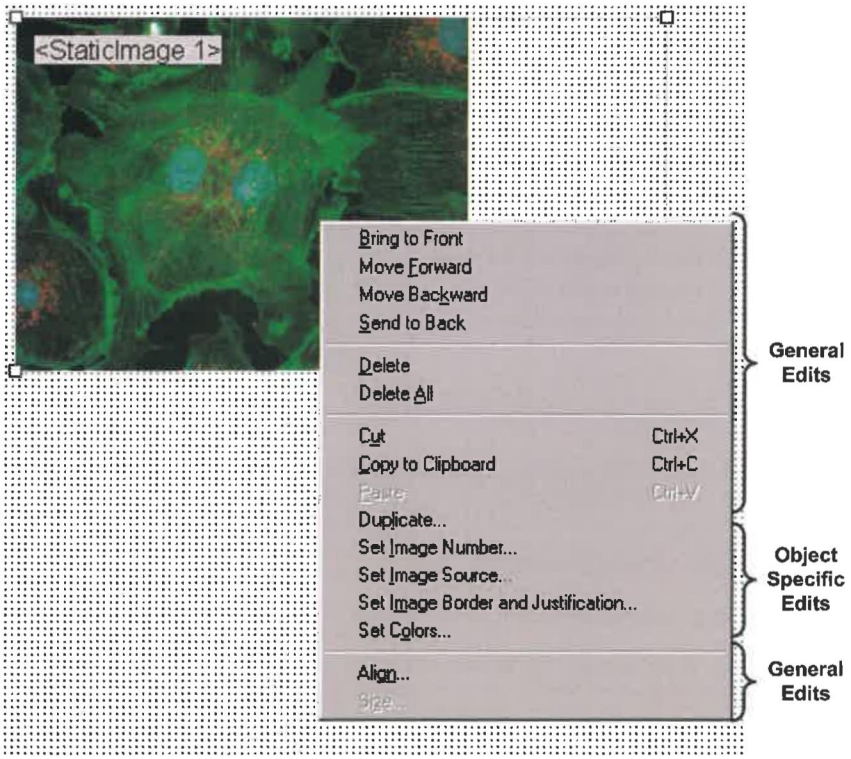
- To size objects, move the cursor either to a point on the object's border or to one of the handles. The cursor changes to a two pointed arrow
  - To resize one dimension of a box, position the cursor on a vertical or horizontal line; click and drag the side to the new size.
  - To proportionally resize a box, position the cursor on a handle; click and drag the box to the new size.
- To position objects, move the cursor over the selected object. The cursor changes to a four point arrow. Click and drag the object to a new location. If an object is selected, you can use the arrow keys to fine tune its position.

Note: When multiple objects are selected, they can be sized and positioned as a single object.

Using Right-click and Double-click Functionality

When you are in report mode, you can use the right-click and double-click mouse functions as editing shortcuts.

- Double clicking on a report object enables you to edit the object's attributes – e.g., the thickness of a line.
- Right clicking (Windows)/ [Ctrl] clicking (Mac) on a report object calls an object specific edit menu that contains both general editing options and attribute editing options specific to that object. Right clicking on an Image object, for example, gives you a list of editing options, as shown at right.
- Right clicking (Windows)/ [Ctrl] clicking (Mac) on the report grid gives you two options: **Paste** an object that you copied or cut; **Delete All** of the objects on the grid.



Refer to the Defining Report Attributes and Editing Report Objects sections for more details.

Defining View Options

The **View** menu provides you with eight options, each of which is described on the following pages.

Zoom Zoom in or out to six different sizes. From the View menu, select Zoom and enter the desired percentage.

First Page Go to the first page in the report.

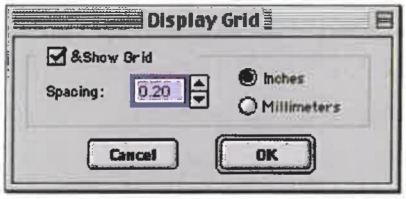
Previous Page Go back one page in the report.

Next Page Go forward one page in the report.

Last Page Go to the last page in the report.

Set Display Grid Size The report definition window includes an adjustable grid (i.e., the dots on the screen) to assist you in placing and aligning report objects on the report page. By default, the grid is turned on. To turn off or modify the grid, follow these steps:

1. From the View menu, select Set Display Grid Size.



   - To turn off the display grid, deselect the Show Grid box.
   - To modify the spacing follow steps two through four.

2. Select either inches or millimeters.
3. Enter the spacing for the display grid. The number that you enter represents the space in inches or millimeters between the dots on the grid.
   - If the measurement is **inches**, enter a number between **.04** and **10**.
   - If the measurement is in **millimeters**, enter a number between **1** and **25**.
4. Click on OK. The display grid appears.

Set Object Grid Size In addition to the display grid, you can define a second grid specific to report objects. The object grid controls the movement of objects as you work with them on the display grid. When you select a report object and move it, the object automatically "snaps" to the nearest line in the object grid, allowing you to more precisely align and arrange the objects on your report page.

1. From the View menu, select Set Object Grid Size.

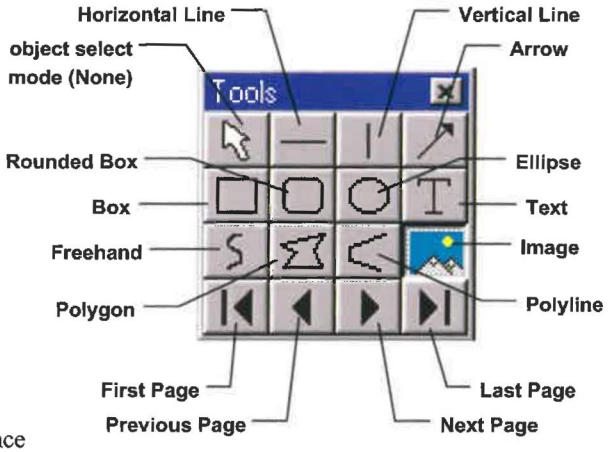
2. If desired, check the Snap to Grid option.
3. Define the spacing for the object grid. In many cases, the object grid and the display grid will have the same spacing. In other cases, you might not want the display grid to be as finely spaced as the object grid (i.e., so the dots do not interfere with the view of the report definition window).
   - If the measurement is **inches**, enter a number between **.04** and **10**.
   - If the measurement is in **millimeters**, enter a number between **1** and **25**.

   The default spacing value for the Object Grid is the last entered value.

4. Click on OK.

Show Tools

Click on the Show Tools option to display a floating palette of navigation buttons and report objects, as shown here.
Refer to the Defining Report Objects section of this chapter for more details.



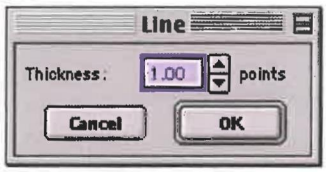
Defining Report Attributes

All of the general style and appearance options (report attributes) can be accessed from the Attributes menu. Report attributes can be defined for an entire report or on an object by object basis (see the Defining Report Objects section below).

- When a report object is selected, the attribute is defined for the selected object(s) only.
- When nothing is selected, the attribute is defined for all future, relevant objects.

The following sections detail each of the report attributes and the report objects that they affect.

Line Thickness



Determine line thickness for the following report objects: **Line**, **Horizontal Line**, **Vertical Line**, **Arrow**, **Box**, **Rounded Box**, **Ellipse**, **Freehand**, **Polygon**, and **Polyline**.

1. From the Attributes menu, select Line Thickness.
2. Enter a Thickness value between **.05** and **10** points.
3. Click on OK.

Colors

Define the foreground and background colors for all objects. Follow these steps:

1. From the Attributes menu, select Colors.
2. Define the **Text/Lines** color. This is the color of the text, the line, or the border around a text/image box. The default foreground color is black. To select a color click on Color.
3. The next window varies, depending on whether you are using a Windows or a Mac system.
   - Windows allows you to select from a palette of colors or enter specific HSL or RGB color values.
   - Mac OS allows you to select from a palette of colors, enter specific RGB, CMYK, HLS, or HSV values, and enter Internet specific colors.
   - Define the **Background/Fill** color. This is the color that appears behind text and in boxes. Lines and images do not display a background color.

Note: In order to define a background/fill color for a Box or a Rounded Box, a fill must be selected. Similarly, in order to define a background/fill color for a text box, the background (see Text Border, Justification, and Background *below) must be set to Opaque.*

Box/Ellipse Fill

Define the appearance of the Box, Rounded Box, and Ellipse objects.

1. Specify whether you want a fill for the object.
   - If you do not want the box to be filled (i.e., you want a transparent box), click on OK.
   - If you want to specify a fill pattern for the box, check the Fill Box/Ellipse. The **Pattern** options appear.
2. Specify a pattern by clicking on one of the seven options.
3. Click on OK.

Image Border and Justification

Determine the line thickness for image borders and define the justification of the image within the display box. This option applies only to the **Image** report object.

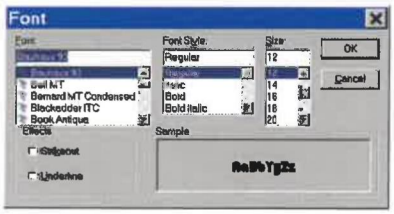
1. To create a border around the image, click on the Border box, and enter a **Thickness** value between **.05** and **10**.
2. Select the **Justification** for the image. This value is the justification of the image within its display box, not the justification on the report page.
3. Click on OK.

Arrow Size and Direction

Specify the size of the arrow head and define the direction that the arrow points.

1. To specify the size of the arrow head, enter a value between **1** and **10**.
2. Specify the direction of the arrow:
   - To draw an arrow starting tail first and finishing with the point (head), select **Tail to Head**.
   - To draw an arrow starting point (head) first and finishing with the tail, select **Head to Tail**.
3. Click on OK.

Text Font



Define the appearance of the text for the **Text**, **Date**, and **Time** objects.

1. Select a **Font**, a **Font Style**, and a point **Size**. The Sample box displays a preview of the font.
2. Define any Strikeout or Underline effects.
3. Click on OK.

Text Border, Justification, and Background

Define the appearance of the text boxes for the **Text**, **Date**, and **Time** objects

1. Specify whether the text box should have a border. To add a border, click on the **Border** box, and enter a Thickness value between **.05** and **10**.
2. Define the **Justification** of the text. This justification applies to the position of the text in the box, not the position of the text on the page.
3. Define the Background. Select either **Transparent** or **Opaque**.
4. Click on OK.

Title Options

Specify how the title of an image will appear on a report. This attribute applies only to the **Image Title** report object. If you have not added an image title to your report, the Title Options do not matter.

- To display only the file name – e.g., Healthy1 – leave both options unselected.
- To display the entire path for an image – e.g., C:\My Images\Cells\Healthy1 – check the **Show Path for File Names** box.
- To display the file name with the file type extension – e.g., Healthy1.jpg – check the **Show Extension for File Names** box.
- To display the file path and the file name with the extension – e.g., C:\My Images\Cells\Healthy1.jpg – check both boxes.

Time Options

Specify whether the **Image Time** report object will include seconds and milliseconds in addition to the hour and minute.

- To display only seconds, check the **Show Seconds** box.
- To display seconds and milliseconds, check both the **Seconds** and **Milliseconds** boxes.

Defining Report Objects

All of the components of your report, including the formatting, text, images, and fields are known as objects. Using the objects, you can specify the information that your report will contain, as well as how the report will work.

- You can, using run-time images, produce reports for images as they are captured. This is a good option for situations where you are capturing many images in one sitting.
- You can produce reports for images that are saved to a SPOT database. Refer to Ch. 9 – Using SPOT Databases for details on creating and modifying SPOT databases.
- You can produce ad-hoc reports for single or multiple saved images.

Before discussing the individual report objects, review the following general procedure for inserting an object into a report:

1. From the Object menu, select the type of item (i.e., Lines, Connected Lines, etc…) that you want to add to the image. The cursor's functionality varies according to whether a report object type (i.e., line, text) is selected:.
   - If a report object type is selected, the cursor changers to a cross-hair icon that is used to add new report objects to the grid.
   - If **None** is selected, the cursor is a an editing tool that is used to select and position objects.
2. Use the mouse to drag the cross-hair to the location where you want to insert the object.
3. Left-click at the insertion point.
   - If you selected a line or a box, hold down the mouse button and drag the line/box to the desired size.
   - If you selected any of the other report objects, the object will appear and you will be prompted to enter the associated information (e.g., static text, the location of an image file, the database field, etc…).
4. Once it appears on screen, you can resize, reposition, and edit an object as needed.
   - **Resize** - Select (click on) the object and drag the handles in the direction of the resize.
   - **Reposition** – Left click and drag the object the new area.
   - **Edit** – Select the object and choose an option from either the Edit menu or the Attributes menu. You can also edit an object by **right clicking** (Windows)/**[Ctrl] clicking** (Mac) on it.

Refer to the table below for descriptions of the SPOT report objects.

None	Select **None** when you want to use the cursor to edit and position the objects in your report.
Lines	**Unconstrained Line** – Add a straight line annotation to the report.
	Horizontal Line - Add a horizontal line annotation to the report.
	Vertical Line – Add a vertical line annotation to the report.
	Arrow - Add an arrow to the report. Either select Arrow from the Object menu, or click on the Tools box button. Left click on the report and drag toward the area that the arrow head will point to.
Connected Lines	**Polygon** – Add a closed polygon to the report. Either select Connected Lines>Polygon from the Object menu, or click on the Tools box button. Left click and release, and then drag the mouse to create a side. Left click again to start a new side. Double click to finish the polygon. The program automatically adds any additional lines needed to close the figure.
	Polyline – Add an open polygon to the report. Either select Connected Lines>Polyline from the Object menu, or click on the Tools box button. Left click and release, and then drag the mouse to create a side. Left click again to start a new side. Double click to finish the polyline.
	Freehand - Add a freehand line to the report.
Shapes	**Rectangular Box** - Add a square cornered box to the report.
	Rounded Box – Add a round cornered box to the report.
	Ellipse - Add an elliptical object to the report. Ellipses are drawn inside of the rectangular area that you define. Ellipses touch the top, bottom, right and left sides of this rectangle.
	Either select Shapes>Ellipse from the Object menu, or click on the Tools box button. To draw an ellipse, left click at the point where you want to position one vertex of the rectangle and drag the cursor to the point of the opposite vertex.

Images

Static Image

A static image is an image that you want to appear on all productions of the report. A logo is a good example of a static image. To define a static image, follow these steps:

1. From the Object menu, select Images>Static Image.
2. Left click at the point on the display grid where you want to insert the Static Image box. As you insert the box, you can also size it by dragging a corner or side.
3. The Static Image box then prompts you to enter the source for the image file. Select from one of the three options:
   - **Currently Open Image**
     If you have more than one image open, select the image from the drop-down list.
   - **Image File**
     A file from disk. Either enter the file name and path, or click on the browse button (to the right of the Image File box) to search for the file. If you select a **sequence** file, specify the image number (i.e., the order in the sequence) in the **Sequence Index** box. Note that, if the **Store Image in Report Template** is selected, you can view the image from within the report template as well as in the report itself. This feature allows you to quickly generate printed reports, without having to first save the report to file and/or run a preview report. Refer to Instant Reports in the Running a Report section of this chapter for details.
   - **Database (Windows)**
     An image file from a SPOT database. In order to use an image from a database, that must be open. If you are not sure of the Image ID, search the database and view the results table – the image ID appears there.
4. Click on **OK**. The static image object (or the actual image if you checked the Store Image in Report Template box) appears on the display grid, where you can move or resize the box.

When you run the report, the image is automatically proportioned to fit the dimensions of the box.

Images (cont.)

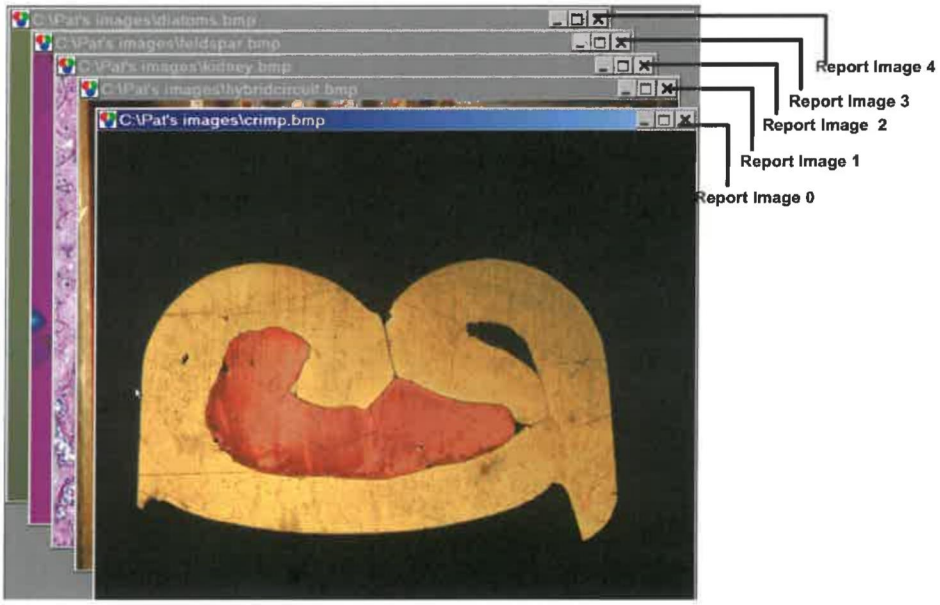
Run-Time Image

A run-time image is an image that is open at the time that you run the report. Run-time image objects allow you to report on images as you capture them, without having to save the images to file or to a database.

To define a run-time image, follow these steps:

1. From the Object menu, select Images>Run-Time Image.
2. Left click at the point the on the display grid where you want to insert the Run-Time Image box. As you insert the box, you can also size it by dragging a corner or side. The Run-Time Image window prompts you to specify the image.
3. Specify which image (if you have multiple open images) will be the run-time image. The entry in the Offset from Top Image Window box defaults to 0. If you have only one image open, click on OK.

   If you have more than one open image, you will need to specify the number for that image. For example, if you have five open images, there are five values, from 0 – 4 that correspond to the images, as illustrated in this cascaded view of multiple image windows:



   If you have multiple open images, selecting a cascade view of the images can help you to determine the proper number. To do this, select the Cascade option from the Window menu.

4. Click on OK. The Run-Time image box appears on the display grid, where you can move or resize the box. When you run the report, the image is automatically proportioned to fit the dimensions of the box.

Images (cont.)

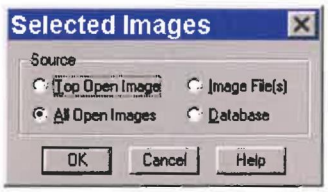
Selected Image

The Selected Image options allows you to select one or multiple images to be included on a report at the time that you run the report. Selected images can be:

- Single or multiple open images
- Single or multiple images from file
- Single or multiple database images

To define a selected image or images, follow these steps:

1. From the Object menu, select Images>Selected Image.
2. Left click on the point in the display grid where you want to insert the selected image.
3. Size and position the image box as desired.
4. Add any additional report objects needed to complete the report.
5. From the File menu, select Run Report.
6. Select the report template either from the **Currently Open Template**, or from **File**.
7. Select the **Output** format. The default choice for running a report is to use the Preview Window, which allows you to view the report before saving or printing it. For more details on the Run Reports options, refer to the **Running Reports** section of this chapter.
8. Click on Run. The Selected Images window appears.
9. Define the Selected Images.
   - **Top Open Image** – The report displays only the top- most image, if multiple images are open.
   - **All Open Images** – The report displays all open images.
   - **Image File(s)** – The report displays the image file or files selected from disk. When you check the Image File(s) option, and click on OK, the Report Images window appears for you to select one or more images to be included in the report. To individually select multiple image files, hold the **[Ctrl]** key and click on each file.
   - **Database (Windows)** – The report displays one or more images from a database. When you check the Database option and click on OK, the Database Search window appears. Add search conditions and ordering criteria, as needed, and click on Search. The report displays the Search Results. Refer to Ch. 9 – Using SPOT Databases for detailed steps on how to define search and order conditions.
10. Click on **OK**.



Text

Static Text

Use the Static Text option to add text that will be the same for all of the images in a report. Typical static text fields include:

- The title of the report
- Report field headings

To define Static Text, follow these steps:

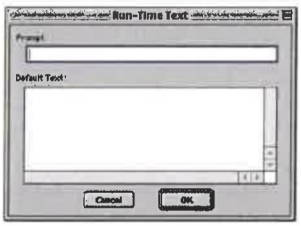
1. From the Object menu, select **Text>Static Text**.
2. Left click at the point where you want to insert the text. The program prompts you to enter the text:
3. After you have entered the text, click on **OK**. The Static Text appears in a box.
4. To edit the text, double-click on the box. The Static Text box reappears for you to modify the text.

Run-Time Text

Use the Run-Time Text option to define the data entry prompts that will appear when you run an image report. For instance, if a static field on your Blood Samples report is Blood Type, you can define a Blood Type? Run-time field that prompts you to enter the blood type for each sample, as the report is run.

To define Run-time Text, follow these steps:

1. From the Object menu, select Text>Run-Time Text.
2. Left click at the point where you want to insert the text. The program prompts you to enter a prompt and default run-time text.
3. Enter the Prompt. When you run a report on an image, you will be prompted to enter information for the prompt.
4. Enter the Default Text, if desired. The Default Text field is useful in cases where the same item is entered for multiple image reports. For example, one lab technician might have drawn the sample for numerous blood samples. In this case, you could enter this technician's name as the Default Text.
5. Click on OK. The object displays on the report grid.
6. To edit the text, double-click on the box. The Run-Time Text dialog box reappears for you to modify the text.



Page Number

Add a page number to a report page.

Page Count

Add a display of the total number of pages in your report.

Record Number

Add the number of the record (selected image/s) to the report. When you run the report, the number for each record is displayed on the page.

Record Count

Add the total number of records (selected image/s) in the report. When you run the report, the Record Count appears on each page.

Report Date

Insert the current date in your report.

Report Time

Insert the time that you ran the report.

Text (cont.)

Image Date
Include the date that an image was captured.

1. After you have inserted an image in the Report, select **Text>Image Date** from the Object menu.
2. Left click at the point where you want the Image Date to appear. The ImgDate box appears on the display grid.

   The number following ImgDate is associated with the image or images in your report. Thus, if the report has more than one image, the program prompts you to define the image that the date should be associated with.
3. Enter the Report Image Number. When you insert images, the program automatically assigns it a number. The first image you insert is 1, the second image 2, etc...
4. Click on OK. When you run the report, the image date displays.

Image Time
Include the time of day that an image was captured.

1. After you have inserted an image in the Report, select **Text>Image Time** from the Object menu.
2. Left click at the point where you want the Image Time to appear.

   The number following ImgTime is associated with the image or images in your report. If the report has more than one image, the program prompts you to define the image that the time should be associated with.
3. Enter the Report Image Number.
4. Click on OK. When you run the report, the Image Time displays.

Note: For the Image Date and Image Time objects, the date/time that appears here varies according to whether the file was transferred across a network, and whether the file is a SPOT database image. Refer to the Image Date/Time section of Ch. 8 for more details.

Image Title
Insert the file path and name that is associated with an image.

To insert the Image Title, follow these steps:

1. After you have inserted an image in the Report, select **Text>Image Title** from the Object menu.
2. Left click at the point where you want the Image Title to appear.

   The number following ImgTitle is associated with the image or images in your report. If the report has more than one image, the program prompts you to define the image that the title should be associated with.
3. Enter the Report Image Number.
4. Click on OK. When you run the report, the file and path for the image displays.

You might have to resize the ImgTitle box to display the entire file path.

Image Memo
Insert the memo that you entered for a TIFF, JPEG, or SPOT database image. Follow these steps:

1. After you have inserted an image in the Report, select **Text>Image Memo** from the Object menu.
2. Left click at the point where you want the Image Memo to appear. The number following ImgMemo is associated with the image or images in your report. If the report has more than one image, the program prompts you to define the image that the memo should be associated with.
3. Enter the Report Image Number.
4. Click on OK. When you run the report, the memo displays.

Image Sequence Index

Insert the number of a specific image when reporting on an image sequence file.

Text (cont.)

Image Sequence Elapsed Time

Insert the elapsed time between two images. When you run a report that contains the Image Sequence Elapsed Time, the program prompts you to specify both the **Reference Image Index Number** and the sequence number of the image you are reporting on. The Reference Image Index number is any number within the image sequence, typically one that precedes the image being reported on. If you specify a number that comes after the image that you are reporting on, the elapsed time will be a negative value.

Database Record ID (Windows)

Include the record number of a database image in your report. The Database Record ID can only be used with run-time or selected images.

To insert the database record ID for a database image, follow these steps:

1. After you have inserted a database image in the Report (either as a run-time image or a selected image), select **Text>Database Record ID** from the Object menu.
2. Left click at the point where you want the record ID to appear. The **DBRecID** box appears on the display grid. The number following DBRecID is associated with the image or images in your report. If the report has more than one image, the program prompts you to define the image that the database record should be associated with.
3. Enter the Report Image Number.
4. Click on OK. When you run the report, the record ID of the database image displays.

Database Field Name (Windows)

The Database Field Name and Database Field Entry objects allow you to run reports that parallel some or all of the information from a SPOT database. Use the Database Field Name object to insert a text/numeric or lookup field from an open database.

1. After you have inserted a database image in the Report (either as a run-time image or a selected image), select **Text>Database Field Name** from the Object menu.
2. Left click at the point where you want the field name to appear. The program prompts you to select the field that you want in the report.
3. Select an entry from the **Field** drop-down list. The drop-down list options are generic i.e., Text/Numeric – 1, Text/Numeric – 2, etc....
4. Click on OK.
5. If the report has more than one image, the Image Number dialog box prompts you to define the image that the database field name should be associated with.
6. Enter the **Report Image Number**.
7. Repeat steps one through seven to add additional database fields to the report.

Database Field Entry (Windows)

The Database Field Name and Database Field Entry objects allow you to run reports that parallel some or all of the information from a SPOT database. Use the Database Field Entry object to insert the data from a text/numeric or lookup field.

To insert a Database Field Entry in your report, follow these steps:

1. After you have inserted a database image in the Report (either as a run-time image or a selected image), select **Database Field Entry** from the Object menu.
2. Left click at the point where you want the field name to appear. The program prompts you to select the field that contains the entry (data) that you want to appear in the report:
3. Select an entry from the **Field** drop-down list.
4. Click on **OK**. The box at right appears.
5. If the report has more than one image, the program prompts you to define the image that the database field entry should be associated with.
6. Enter the **Report Image Number.**
7. Click on **OK**. When you run the report, the database field entry displays.
8. Repeat steps one through seven to add additional database field entries to the report.

User Name

User Name – Add the user's name to the image. This defaults to the user who is logged on the computer.

Editing Report Objects

Once you have created a report, you can edit the report objects as needed. There are three basic ways to edit a report object:

- **Use the Attributes menu** - The Attributes menu, as noted in the Defining Report Attributes section of this chapter, allows you to either set the attributes for adding specific types of objects, or to select existing objects and modify them as needed. Refer to the Defining Report Attributes section for more details.
- **Use the Edit menu** - The Edit menu contains general editing features such as Undo, Copy, Cut, Send to Back, etc…
- **Right-click (Windows)/[Ctrl] click (Mac) on the report object** - Right clicking/[Ctrl] clicking on a report object brings up a customized editing menu that contains both general editing commands and the attributes that can be edited for that object. Refer to the Working in the Report Mode section for more information on the right click function.

The Edit Menu

The Edit menu provides general editing features for arranging, copying, and sizing report objects. Edit menu options are active only when a report object is selected. The following sections describe each of the Edit menu options. As is explained in the sections below, not all of the options are available at all times.

Undo Undo the last action. The Undo feature allows you to undo up to ten actions.

Bring to Front Bring a selected object in front of all other objects.

Move Forward Bring a selected object one layer forward.

Move Backward Send the selected object one layer back.

Send to Back Send a selected object behind all other objects.

Select All Select all report objects.

Delete Delete the selected object(s).

Delete All Delete all of the objects in the report.

Copy to Clipboard Copy the selected object(s) to the Windows clipboard. You can then paste the object(s) into:

- A new SPOT report
- A different location in the same report

This feature is particularly useful when you are creating a new report and want to use objects from an existing report.

Cut Cut the selected object(s) for pasting into a new report or to a new location in the existing report.

Paste Paste the object that you cut or copied to its new location.

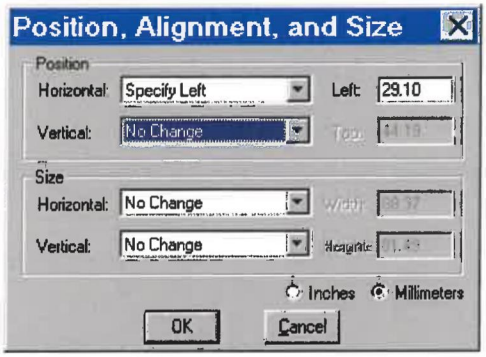
Duplicate

Create additional copies of a report object. To duplicate a report object, follow these steps:

1. Select the object(s) that you want to duplicate.
2. Select Duplicate from the Edit menu. The program prompts you to enter the number of duplicate copies.
3. Enter the number of copies, and click on OK.
4. The duplicate object(s) appear at the top of report window.

Set Position, Alignment, and Size

The **Set Position, Alignment**, and **Size** option allows you to specify precise sizing and position values for single or multiple selected report and annotation objects.



The **Position** options enable you to:

- Align top/bottom edges of multiple objects.
- Align left/right edges of multiple objects.
- Align the centers of multiple objects.
- Center an object(s) on the page.
- Enter exact values in inches or millimeters to position the object.

The **Size** options enable you to enter:

- Exact width/height in inches or millimeters for one or more objects.
- Set the width/height dimension for multiple objects the to smallest or largest object's value in inches or millimeters.

Add Page

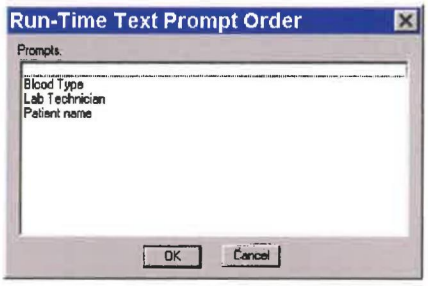
Add a page to your report. When you add a page, the program prompts you with the Add Page dialog box:

1. Specify whether the page that you want to add will be inserted Before or After a specified page.
2. Specify the Page Number that the additional page will precede or follow.
3. Click on OK.

Delete Page Delete a page from your report. When you select the Delete Page option, the program prompts you to specify the page number.

Add Static Images Add a static image object to the report. Refer to the Static Image object description in the Defining Report Objects section above for details on this process.

Order Run-Time Text Prompts When you run a report with multiple run-time text prompts (see the Defining Report Objects section), the prompts, by default, appear in the order that you defined them. Use the Order Run-Time Text Prompts option to rearrange the order as needed.

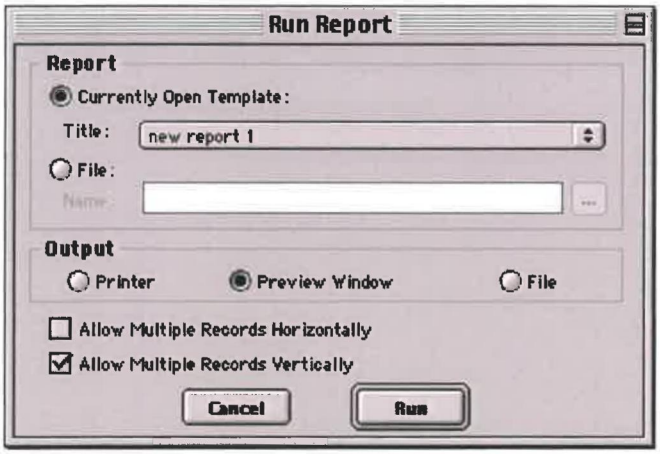


To re-order the run-time text prompts for a report, follow these steps:

1. From the Edit menu, select Order Run-time Text Prompts. The current order of the run-time text prompts displays.
2. Click and drag the prompts to rearrange them in the desired order.
3. Click on OK. When you run the report, you will be prompted to enter the run-time text in the newly defined order.

Running a Report

To run a SPOT report, follow these steps:



1. From the File menu, select Run Report. You can either run an open report or a report from file:
   - **Currently Open Template**
     If more than one report template is open, select from the drop-down list.
   - **File**
     Either enter the file name and path for the report that you want to run, or click on the Browse button to the right of the Name box to search for the report file.
2. Select the output format for the report.
   - **Printer** –The report is sent directly to the printer. The program first prompts you to select a printer/define print settings. Refer to Ch. 11 – Printing Images for details on printing from the SPOT program.
   - **Preview Window** –The report is displayed on screen, where it can be viewed, printed, and/or saved. If you have not run any previous reports, this is the default option. Otherwise, the program defaults to the output format for the last run report.
   - **File** – The report is saved directly to a file directory as a **Run Report (.rpd)** file. The program prompts you for the file location. The report can be opened for viewing or printing at a later time.

   The output options are discussed in more detail in the sections below.

3. Specify the **Allow Multiple Records Horizontally** and **Allow Multiple Records Vertically** options. When a report contains multiple Selected Image objects, SPOT separates each image with a page break: the report displays only one image per page. The **Allow Multiple Records Horizontally** and **Allow Multiple Records Vertically** options allow you to display multiple images on a page for cases such as a report on a database or a sequence image file.

   - To display multiple images in a row, check **Allow Multiple Records Horizontally**.

   - To display the multiple images in a column, check **Allow Multiple Records Vertically**.

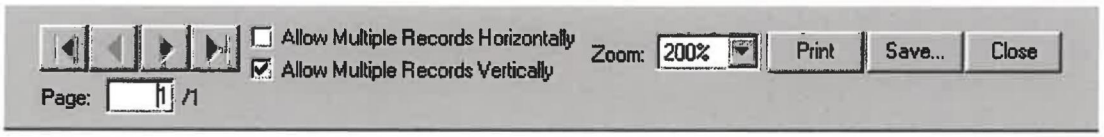
   - To display the images in rows and columns, check both options.

   In addition to selecting these options from the **Run Report** dialog, you can select them from the **Preview** window (see **Previewing a Report** below)

4. Click on Run. The prompts that you see depend on the type of report objects that you defined. Refer to the Defining Report Objects section in this chapter for more information.

Previewing a Report

When you run a report in the Preview Window, the report appears with a navigation toolbar at the bottom of the preview window, as shown here:



From left to right, the toolbar buttons are as follows:

- **Navigation arrows** - Move forward or backward in a multi-page report, or to the first page or last page in the report.
- **Page display** – See which page of the report you are viewing.
- **Allow Multiple Records Horizontally** – Display multiple selected images in a row.
- **Allow Multiple records Vertically** – Display multiple selected images in a column.
- **Zoom** – Zoom in or out on the report. There are six options.
- **Print** – Print the report without saving it.

- **Save** – Save the report as a Run Report (.rpd) file.
- **Close** – Close the report preview without printing or saving it.

Printing a Report

When you run a report with an output to Printer, the program prompts you to enter print information:

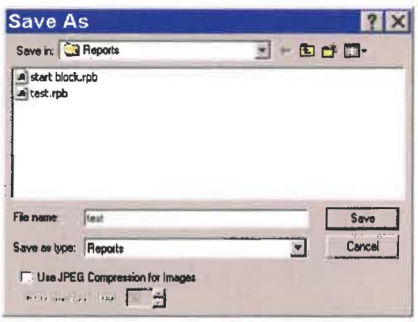
Follow these steps:

1. Select the printer.
2. Click on Properties to define the printer's properties, if needed.
3. Specify the page range if you are not printing an entire report.
4. Specify the number of copies.
5. Click on OK.

Saving a Report to File

When you run a report with an output to File, the program prompts you to save the file. Follow these steps:

1. When saving a new report (**.rpd** file type), SPOT prompts you with the default file location – i.e., **C:SpotCam\Reports** folder (Windows) or **Users\Shared\SPOT Files\Reports** (Mac) . Either save to the default location or specify a new location. If you plan to save many reports without compressing the image files, you should save them to a Jaz drive or to a network drive.



> *Note: Mac users will see the* **.rpd** *file type extension only if the* **Add Extensions to File Names on Save** *option is selected on the Preferences window .*

2. Enter a file name for the report.
3. Define the JPEG Compression for the image(s) in the report. The **Use JPEG Compression for Images** box is checked as a default. To turn off JPEG compression, deselect the box. If you are using JPEG Compression, enter a number between one and 100.
   - A value of 1 is the most compressed. File size and image resolution are decreased to the maximum extent.
   - A value of 100 is non-compressed. File size remains large and the image resolution remains high.
4. Click on Save. You can now use the SPOT program to open and view the report at a later time.

Instant Reports

To quickly preview and print a simple report, without first running a preview report or saving the report to file, use the new **Store Image in Report Template** feature. The Store Image in Report Template feature allows you to view a Static Image report object in the report template: you do not need to run a preview report to see what the final printed product will look like.

Follow these steps:

1. Open a new report template. From the File menu, select **New Report**.
2. Add a report image that to the template. From the **Object** menu, select **Images>Static Image**. Left-click and drag the cross-hairs cursor to size and position the Static Image box. When you release the button, the **Static Image** window appears.
3. Select **File** for the Image Source. Click on the button to the right of the **Name** box to select an image.
4. If you have selected a sequence file, specify the **Sequence Index** – i.e., the number of the image in the sequence.
5. Ensure that the **Store Image in Report Template** box is checked.
6. Click on **OK**. The Static Image appears in your report, where it can be sized, positioned, or annotated as needed.
7. Add additional static images as needed. Follow steps one through seven.
8. From the File menu, select **Run Report**.
9. For the Output Destination, select **Printer**.
10. Click on **Run**.

Distributing Reports

If you intend to distribute SPOT image reports to people who do not have the SPOT program, we recommend that you purchase Adobe Acrobat. Using Acrobat, you can convert the SPOT report (.rpd) files to PDF files, which can be distributed to users who do not have the SPOT software. They can view the reports with the free Acrobat Reader software, which is included on the SPOT software CD. The latest version of the Acrobat Reader can also be downloaded from the Adobe's web site www.Adobe.com.

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Ch. 11 - Printing Images

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QUICK REFERENCE:

- Print a single image... p. 204
- Print multiple images on one page... p. 205
- Add images from file and/or SPOT database images for printing... p. 205
- Arrange how multiple images appear on the page... p. 206
- Print an image sequence... p. 206

Introduction

The printing features in the SPOT program fall into two categories:

- **Printer Setup** - The standard Windows dialog boxes that are used to specify:
  - Printer and setup
  - Page setup
  - Orientation
  - Paper size and source.
- **Print** - The SPOT printing options allow you to print one or multiple diagrams on a page.

Printer Setup

After you have installed and setup your printer, the procedure for setting up and configuring a printer in SPOT is the same as in any other Windows program.

Note: Because the information contained in this section is standard Windows functionality, the options are not discussed in detail. For more information on Windows printing features, refer to the Windows on-line help.

To choose and configure a printer, follow these steps:

1. **Choose a printer.** From the File menu, select Printer Setup. The Page Setup window appears. To select the printer you want to use, click on the Printer button at the bottom of the window. The program prompts you to select a printer.

2. **Modify the default settings of the printer, if necessary.**
   Click on Properties. From this point you can modify printer specific options such as color settings, fonts, graphic settings, etc... The printer properties window and the available options will vary according to the printer that you are using. Refer to the on-line help or your printer's manual for more information. When you finish entering the property settings, click on **OK**.

3. Click on **OK**. The program returns you to the original Page Setup window.

4. **Define the page setup that you want to use for the image(s).**
   This includes the page orientation (portrait or landscape), paper size, paper source, and margins.
5. **Save the page layout specifications that you entered.**
   Click on OK.

Note: You can also access the Page Setup screen from the Print screen (i.e., From the File menu, select Print, and click on the Setup button.

Print

After you have defined the printer and page layout, you are ready to print. With the SPOT software, you can print a single image to a page or you can print multiple images on a page for comparison or display.

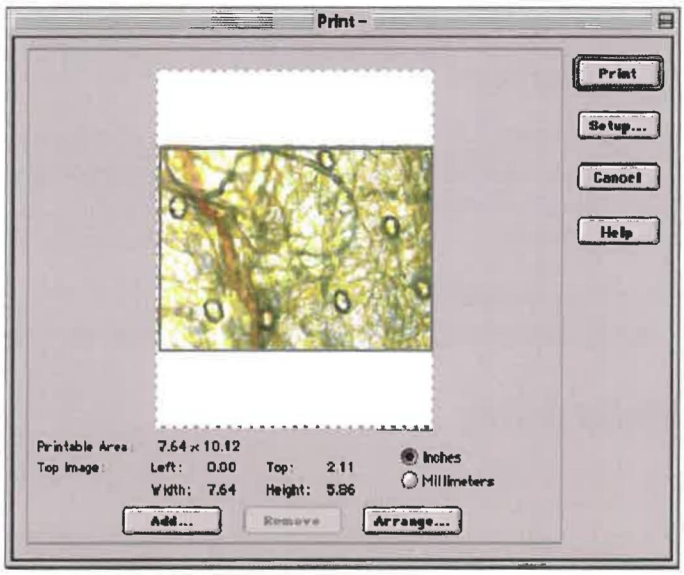
Printing a Single Image

To print a single image from the SPOT software, follow these steps:

1. Either select Print from the File menu, or click on the Print toolbar button. The Print window appears with a preview of the active image.

   The Print window displays the following information:

   – **Printable Area:** The printable dimensions of the page.
   – **Top Image:** The position and size of the top-most image (if more than one image will be printed on the page). They are:
   - **Left** - The distance between the left side of the printable area and the left side of the image.
   - **Top** - The distance between the top of the printable area and the top of the image.
   - **Width** - The width of the image.
   - **Height** - The height of the image.



2. Check either **Inches** or **Millimeters** to specify the units of measurement.
3. Adjust the size of the image:
   a) Position your cursor on a side or a corner of the image. The cursor turns into a two-ended arrow.
   b) Using the two-ended arrow cursor, drag a side or corner to enlarge or reduce the image. The resized image maintains the height to width ratio of the original image.
4. Adjust the position of the image.
   a) Position the cursor on the image. When on the image, it appears as a four pointed arrow.
   b) Left click and drag the image to the desired location.

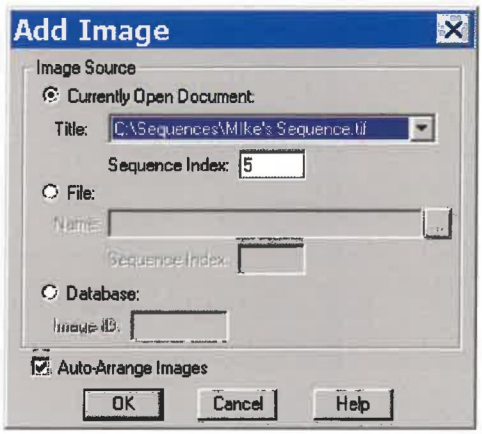
5. Click on the Print button. The Printing dialog box displays the job. To stop the print job, click on the Abort button.

Printing Multiple Images on a Page

The SPOT program also enables you to print multiple images on a single page. The additional images do not need to be open; you can add additional images from a file directory or a SPOT database.

To add and arrange multiple images for printing, follow these steps:

1. From the File menu, select the Print option. The currently open, selected image appears in the Print window.
2. Add an image to the page for printing. Follow these steps:
   a) Click on the Add button. The Add Image window appears.
   b) Select the image that you want to add. Images can be added from one of three places:



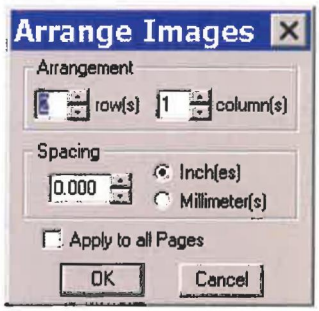
   - From a **Currently Open Image** – Add one or all of the currently open images. Select either the desired image or **<All Images>** from the Title drop-down list. Ensure that you enter the **Sequence Index** (i.e., the number of the image in the sequence) if you are adding an image from a sequence file.
   - From an **Image File** – Add a file from disk. Check the **Image File** option, and click on the square next to the box. Browse for the desired image, and click on Select. Ensure that you enter the **Sequence Index** (i.e., the number of the image in the sequence) if you are adding an image from a sequence file.
   - From a SPOT **Database** – Add an image file from an open SPOT database. To add an image file from a SPOT database, you must know its Image ID number. After you have determined the number, check the **Database** option, and enter the ID number.

   c) Set the auto-arrange option. If you check the Auto-Arrange Images box, the SPOT program automatically sizes and positions the images to fit on the print page.

   d) Click on OK. The program adds the images to the print page and returns to the Print window. The images appear in the preview box.
      - **If you selected the Auto-Arrange Images option**, the images will be arranged in a print-ready format. Click on Print to print the page.
      - **If you did not use the Auto-Arrange Images option**, the images will be stacked on top of each other for you to arrange. Go to step 3 for details on manually arranging the images.

   To add additional images, repeat step 2.

3. Arrange how multiple images will appear on the page. Follow these steps:



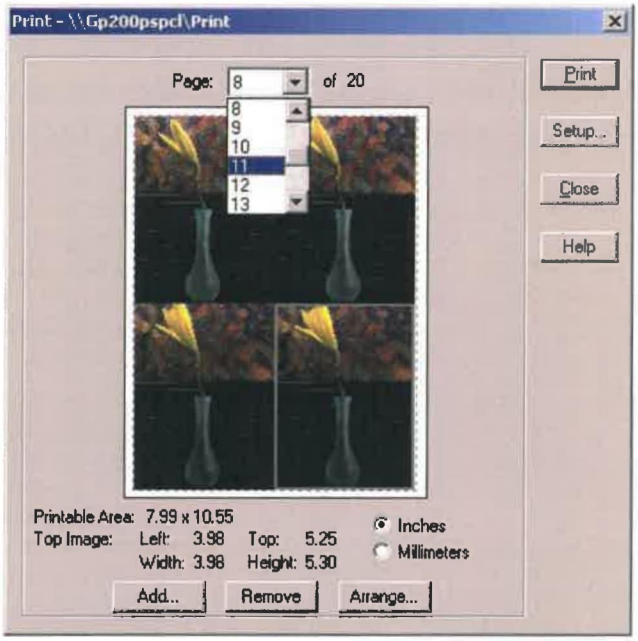
   a) Click on the Arrange button. The Arrange Images window appears.

   b) Select the number of row(s) and column(s).

   c) Enter the Spacing between the images on the print-out in inches or millimeters.

   d) Select **Apply to all pages** to make the arrangement applicable to every page of a multi-page report.

   e) Click on OK. The Print screen reappears with a preview of your print arrangement. The screen capture at right illustrates four images arranged in a two row, two column format.



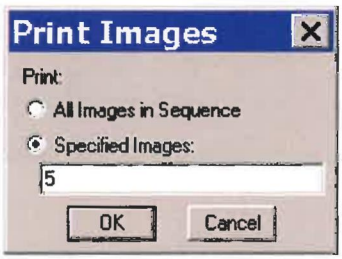
   The SPOT program automatically resizes images to fit on the page, so you can add multiple images without having to resize them. For example, if you add five images, the preview will show six images (the original and five added) of equal size, distributed proportionally across the page.

> *Note: You can reposition and resize multiple images on the preview page, just as you can with a single image.*

4. From the print screen, you can:
   - Click on **Print** to print the page.
   - **Add** an image as described in step two.
   - Delete an image by selecting it and clicking on **Remove**.
   - Reposition and/or resize the images as described in the previous section.
   - Use the **Page** drop-down list to navigate to a different page view (when printing multiple pages.

Printing Image Sequences

The procedures for printing sequential images in the SPOT software are identical to those described in the **Printing Multiple Images on a Page** section (see above)., with one exception. When you select the **Print** option for a sequential image file, the program prompts you to specify the image(s) in the sequence that you want to print, as shown at right.



- If you select **All Images in Sequence** and click on OK, the program prompts you to specify the number of images per page.
- If you select **Specified Images**, you can select either a single image or multiple images. Separate multiple images with commas and/or dashes – e.g., **1, 2, 3**; **1-3**; or **1, 2, 3-6**.

Ch. 12 – Using Other Windows Imaging Applications in Advanced Mode

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QUICK REFERENCE:

- Set up the camera to work with another imaging application (define the TWAIN interface)... p. 208
- Define an image setup from the SPOT TWAIN interface... p. 2100
- Preview an image in the SPOT TWAIN interface.... p. 211
- Transfer a captured image to a TWAIN compliant application... p. 211
- Capture an image and have it open in the TWAIN application... p. 211

Introduction

The SPOT software is TWAIN compliant. The TWAIN interface, as an alternative to the stand-alone SPOT software, allows Windows users to use other imaging programs (e.g., Photoshop or Image Pro) to view, and edit images that were captured using the SPOT camera and software. For example, using the TWAIN interface and the SPOT camera, you can capture an image from within Photoshop and then transfer the image into PhotoShop to be edited and saved. The following chapter details the use of the SPOT TWAIN interface via Adobe Photoshop 5.0.

Note: Adobe Photoshop was selected as the example to illustrate SPOT's TWAIN features because it is one of the most commonly used "TWAIN capable" image editing programs.

This chapter is divided into two sections.

- Preparing to Take a Picture
- Taking a Picture

Note: Because the image setup and image capture functionality is identical in the stand-alone and TWAIN interface versions of the software, these sections reference other chapters in the manual.

Preparing to Take a Picture

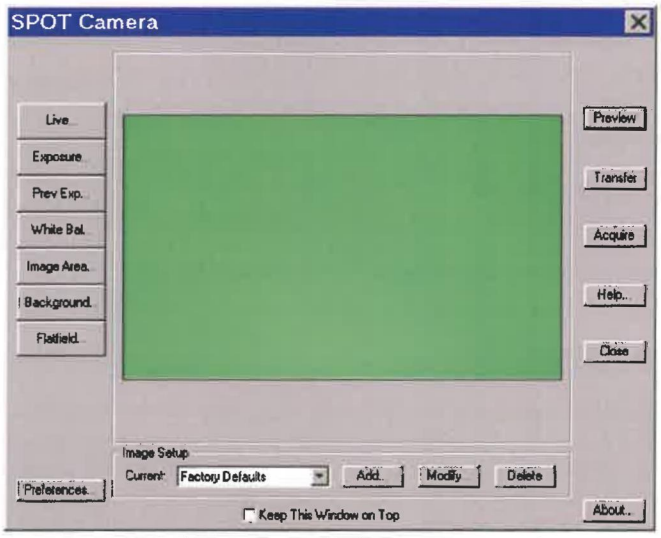
The TWAIN interface provides you with the same image setup options as the stand-alone version of the SPOT software. There are two main tasks in preparing to capture an image.

- Establishing the TWAIN Interface
- Defining an Image Setup

Establishing the TWAIN Interface

To use the SPOT camera and software via Photoshop, establish the TWAIN interface by defining the SPOT software as the default TWAIN source Follow these steps:

1. Open Photoshop.
2. Define the source of the program that you want to interface with (in this case the SPOT software). From the **File** menu, select **Import>Select TWAIN-32 Source**. The Select Source window appears.
3. Select **SPOT Insight Camera (Advanced)** Camera, and click on Select. The SPOT software is now the default TWAIN source.



> *Note: To work in the Basic mode TWAIN interface, select* **SPOT Insight Camera (Basic)**. *Refer to Ch. 13 – Using Other Windows Imaging Applications in Basic Mode.*

4. From the File menu in Photoshop, select **Import** and **TWAIN_32**. The SPOT Camera window appears within Photoshop, as shown at right.

Refer to the next sections, Defining Live Image Preferences and Defining Image Setups, for details on how to define image setups from the TWAIN interface.

Defining Live Image Preferences

The TWAIN window includes a **Preferences** button, which allows you to define live image viewing and exposure preferences. This includes the following options:

- Always Show Full Chip
- Maximum Exposure
- Do Post Processing On

Each option is discussed below.

Always Show Full Chip

Always display the full chip in live image mode. If you select a region, it will be displayed in a rectangular outline within the full-chip view.

Maximum Exposure

The Maximum Exposure option helps you to avoid unnecessarily long exposures when viewing a live image. Consider the following situation: When you change from a lower resolution objective to a higher resolution objective, the camera detects a brief interval of blackness and compensates by modifying the exposure time to maintain a constant brightness level. This results in you having to wait for the camera to compute a lengthy and incorrect exposure for the black interval. Using the **Maximum Exposure** option, you can limit this exposure time. For most cases, Diagnostic Instruments recommends a value of 0.5 seconds. Similarly, the Live Image Maximum option can also be used in cases where the microscope's light path selector is incorrectly set.

Post Processing

Specify whether to apply the Post Processing options for the current Image Setup to images captured from the live image view.

Transferred Images

- *If the Transferred Images box is selected,* the Transfer option applies the Noise Filter, Color Enhancement, and Image Size Double options as specified for the current Image Setup.
- *If the Transferred Images box is unselected,* the Transfer option ignores the Post Processing options for the current Image Setup, with one exception: if the Chip Defect Correction option is selected for the current image setup (SPOT and RT cameras only) , the corrections will appear in the image capture, regardless of the Transferred Images preference.

Snapped Images

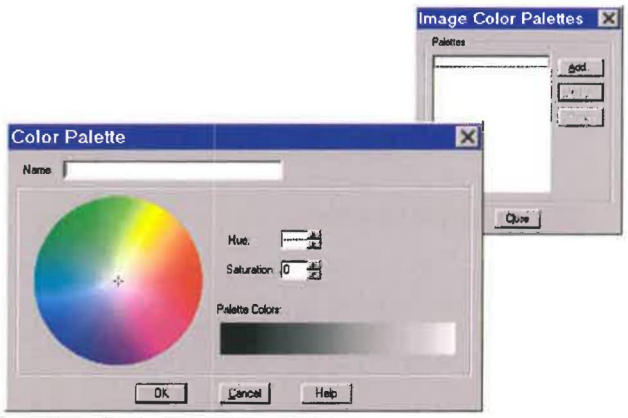
- *If the Snapped Images box is selected,* the Snap option uses all of the Post Processing options from the current Image Setup, except for Background Subtraction and Flatfield Correction.
- *If the Snapped Images box is unselected,* the Snap option ignores the Post Processing options for the current Image Setup, with one exception: if the Chip Defect Correction option is selected for the current image setup (RT and SPOT cameras only), the corrections will appear in the image capture, regardless of the Snapped Images preference.

Palettes (Insight B/W)

The Palettes option allows you to define new palettes and edit existing palettes.

SPOT color palettes allow you to apply specific hue and saturation values to monochrome (8 bit or 12 bit) images. Palettes are useful in fluorescence applications where a sample is fluorescing at a single wavelength, and you want the acquired image to have the correct color. For such a case, you could create a palette to represent the color of the fluorescent dye and then apply that palette to the captured image. There are two ways to apply a palette to an image:

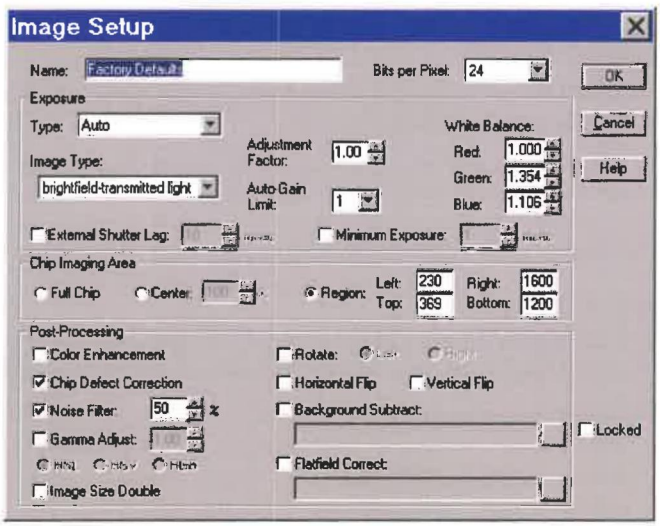
- You can associate a palette with an Image Setup, as discussed in Ch. 5 - Preparing to Take a Picture.
- You can apply a palette to a captured image by selecting the **Set Palette** option from the **Edit** menu. Refer to Ch. 8 – Viewing, Editing, and Annotating Images for more details.



To define a new palette, follow these steps:

1. From the **Setup** menu, select **Palettes**.
2. Click on **Add**. The Color Palette dialog appears as shown here:
3. Either use the **Hue** and **Saturation** selection lists or move the cross-hairs on the color wheel to enter the values
   - **Hue** is the designation for the actual color, as distinguished from others in the color spectrum. Hue is derived from the color wheel and is expressed in the color's angular location (in degrees) on the wheel. (e.g., 0° to 360°).
   - **Saturation** is the proportion of perceived pure hue in the color. Saturation (in the SPOT software) is measured on a numerical scale of 0 to 100, where zero is pure gray and 100 is pure hue (e.g., day glow).
4. Click on **OK**.

Defining Image Setups



An image setup serves two crucial purposes:

- It defines the way a picture will be taken.
- It defines the way the program automatically modifies a picture after it is taken.

Image setups are defined to optimize the picture taking process for different types of images or microscopy techniques. They can be created, saved, and recalled as needed. To define a new image setup, you must also select one of 14 pre-defined image types as a template for defining the new setup. The 14 pre-defined image types (in addition to the Factory Defaults setting) correspond to commonly used microscopy techniques. Refer to Ch. 5- Preparing to Take a Picture for a table of the different image types. Pre-defined image types are indicated by brackets around the title, while the user defined image setups appear without brackets.

- To modify the current image setup, click on the **Modify** button.
- To delete an image setup, select the image setup from the list, and click on the **Delete** button.
- To define a new image setup, click on the **Add** button. The New Image Setup window appears with a list of pre-defined and user-defined templates (image types). Pre-defined image types are indicated by brackets < >. Select a template, and click on OK.

When you add a new image setup or modify an existing setup, the Image Setup window appears, as shown at right. Refer to Ch. 5 – Preparing to Take a Picture for a detailed explanation of the Image Setup options.

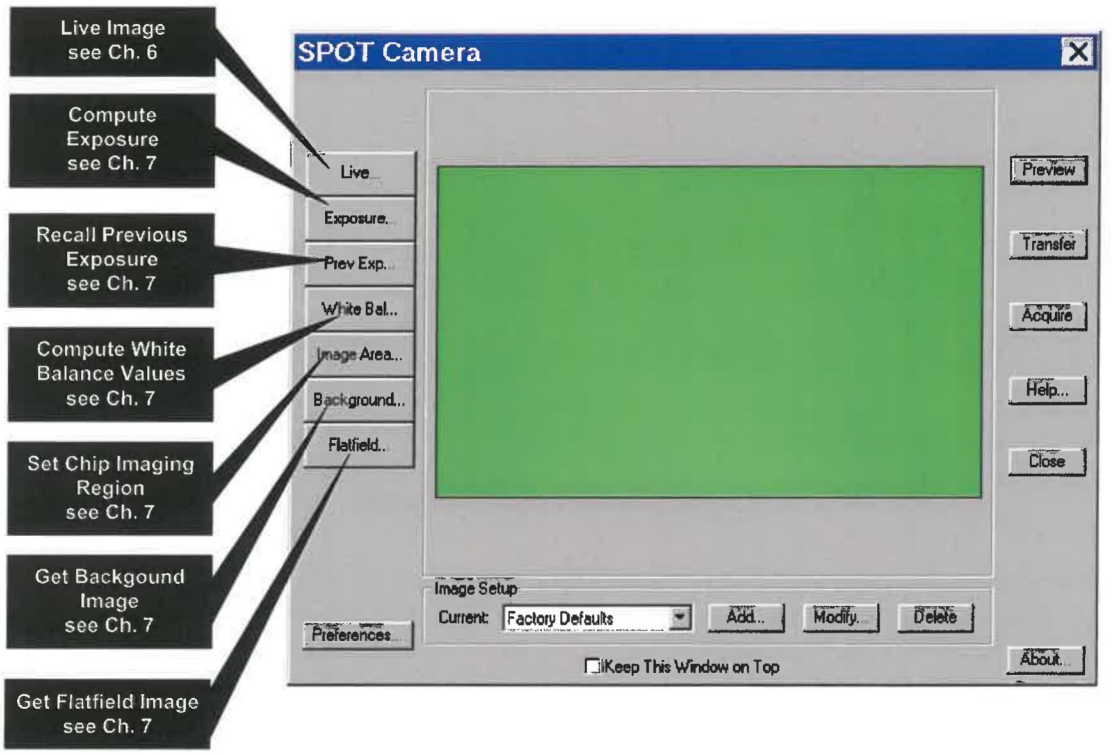
Insight B/W	Insight B/W camera users will not see the fluorescence – red, fluorescence – green, and fluorescence – blue Image Type options.

Taking a Picture

From the SPOT TWAIN interface, you can perform both initial image captures (i.e., for computing exposure, white balance, etc…) and final image captures.

Capturing Initial Images

The toolbar at the left of the SPOT Camera window enables you to quickly access the functions that are used prior to the capture of the final image.



Insight B/W Insight B/W camera users will not see the Compute White Balance Values toolbar button.

Capturing Final Images

From the SPOT TWAIN interface, you can capture an image in two different ways. You can either preview the image and then transfer it to Photoshop, or you can Acquire the image, which sends the captured image directly to Photoshop.

- **Preview** – Capture an image and preview it within the SPOT TWAIN window.
- **Transfer** – Transfer a captured image to Photoshop.
- **Acquire** - Capture an image and have it automatically transfer to (open in) Photoshop.

The **Keep This Window on Top** option allows you to preview and transfer or acquire multiple images without having to re-open the SPOT window.

- If the Keep This Window on Top box is checked, the SPOT window remains open.
- If the box is not checked, the SPOT window automatically closes after each image is transferred or acquired.

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Quick Reference:

- Set up the camera to work with another imaging application (define the TWAIN interface)... p. 214
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Introduction

The SPOT software is TWAIN compliant. The Basic TWAIN interface, as an alternative to the stand-alone SPOT software, allows Windows users to use other imaging programs (e.g., Photoshop or Image Pro) to view, and edit images that were captured using the SPOT camera and software. For example, using the Basic TWAIN interface and the SPOT camera, you can capture an image from within Photoshop and then transfer the image into Photoshop to be edited and saved. As with the stand-alone version of the SPOT software, the TWAIN interface can be used in both Basic and Advanced modes. The following chapter describes only the **Basic TWAIN** Interface. .

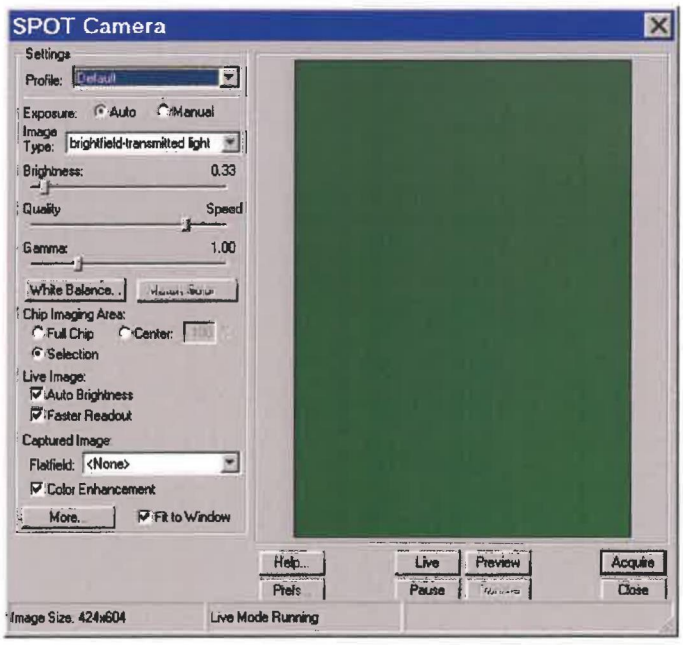
Establishing the TWAIN Interface

To use the SPOT camera and software via a TWAIN compliant imaging application (e.g., Photoshop, Image-Pro, etc…), you must first set the SPOT software as the default TWAIN source.

Follow these steps:

1. Open the TWAIN compliant application e.g., Photoshop, Image-Pro, etc…
2. Specify the source of the program that you want to interface with (i.e., the SPOT software). The procedure for this step will vary according to the software you are using, but you should see a list of TWAIN devices. In many cases, the application asks you to specify a scanning device.
3. Choose **SPOT Insight camera (Basic Mode)**. This establishes the SPOT software as the default TWAIN source.
4. Open the TWAIN interface. Again, this step varies according to the software you are using. The SPOT Camera window appears within the application, as shown at right.

Refer to the next sections for descriptions of the available options on the TWAIN interface.

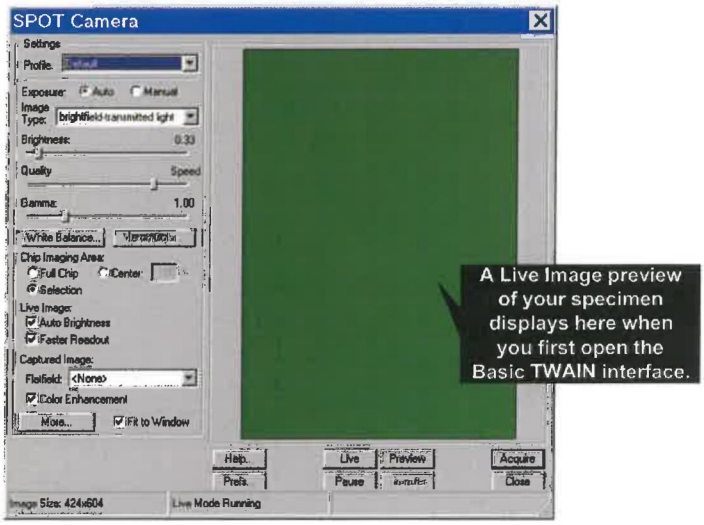


Working in the Basic TWAIN Interface

The Basic TWAIN interface is a one-stop point for previewing and capturing images. For example, using the Basic TWAIN interface, a typical image capture session might involve the following steps, as illustrated below:

Preview your specimen

Use the live image as a point of reference to focus and position the image and to scan for areas of interest.



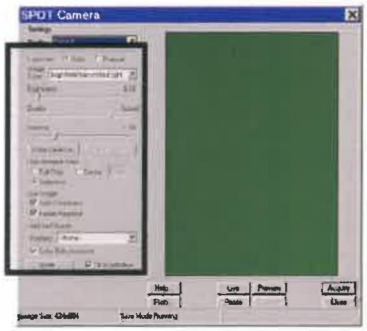
Create a Profile

Profiles allow you to create, modify, and reuse specific exposures settings that are tailored to your image capture needs. To create a new profile, right click on the Profile box.

Settings
Profile:
Default
Add...
Save
Rename...
Delete
Exposure:
Auto
Image Type:
brightfield-tra
Right-clicking on the Profile box gives you options to create and modify Profiles. When you select "Add," you are prompted to name the new Profile. And, as you modify view and capture settings, they are saved to that Profile.

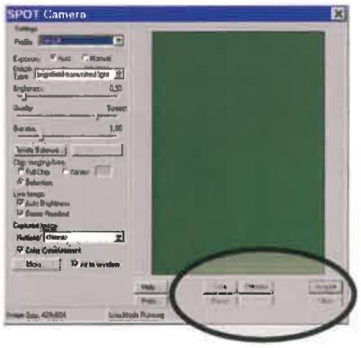
Define Settings

Define exposure settings for live and captured images. Each of these settings are discussed later in this chapter.

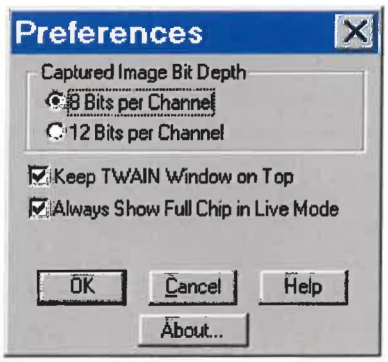


Capture an Image

All of the image capture options are located on the bottom portion of the window, as illustrated here



Defining Preferences



In the Basic TWAIN Interface, you can define three capture/view preferences. Click on the **Prefs** button to open the **Preferences** window (see right). Each option is described below:

Captured Image Bit Depth
This option enables you to specify a different image bit depth when saving an image captured in the SPOT program.

- **8 Bits per Channel** – The 8 bits per channel option provides 256 gray levels per color:
  - With Insight B/W cameras, captured images are 8 bits per pixel monochrome.
  - With Insight Color cameras, captured images are 24 bits per pixel RGB color.

  This option results in smaller, more manageable image sizes, and is best for images that do not require extensive modification.

- **12 Bits per Channel** – The 12 Bits per channel option provides 4,096 gray levels per color.
  - With Insight B/W cameras, captured images are 12 bits per pixel monochrome.
  - With Insight Color cameras, captured images are 36 bits per pixel RGB color.

  This option reduces banding problems that can result with modified images, but also results in larger file sizes.

  When saving 12 bit per channel images, SPOT prompts you to select either 12 bits per channel or 16 bits per channel. If you use Adobe's Photoshop for image editing, you should select the 16 bit per channel option because Photoshop cannot read 12 bit per channel images.

Note: The SPOT Insight camera captures all images using 10 bits per channel (color). The SPOT software immediately converts the captured image to either 8 bits per channel (color) or 12 bits per channel (color), depending on the **Captured Image Bit Depth** *that you select.*

Keep TWAIN Window on Top
Checking this option ensures that the TWAIN window remains the top-most window when working in multiple applications. This allows you to preview and transfer or acquire multiple images without having to re-open the SPOT window. If the Keep This Window on Top box is checked, the SPOT Camera window remains open. If the box is not checked, the window automatically closes after each image is transferred or acquired.

Always Show Full Chip in Live Mode
Checking this option ensures that you will always see the Full Chip view when viewing a live image. Keep in mind that the selection that you make here determines how the live image displays when viewing a region of the chip. Refer to the **Chip Imaging Area** section for more details.

F-Keys

The following function keys are available as shortcuts in the Basic TWAIN interface:

Function Key	Action	TWAIN Interface Option
[F 4]	Restart the live image view.	Live
[Alt] + [F 4]	Close the SPOT Camera window.	Close
[F 9]	Capture an image and automatically transfer it to the imaging application.	Acquire
[Ctrl] + [F 10]	Compute white balance values.	White Balance
[Alt] + [F 10]	Compute white balance values without displaying the informational dialog.	*Not available from the window*

Using the Live Image to Define Image Capture Settings

When you open the Basic mode TWAIN Interface, the Live Image window is, by default, running, as indicated by the **Live Mode Running** message in the status bar. The settings options in the TWAIN interface window enable you to optimize the live image view, and then capture an image using these settings. In the TWAIN interface, you can:

- Create profiles to use as pre-defined image capture settings.
- Modify exposure settings
- Define a specific portion of the chip to be used in the image capture.
- Modify the screen orientation of the specimen.

The following sections describe all of the Live Image/Image Capture Settings.

Profile

The **Profile** option allows you to create and re-use sets of live image/image capture settings. When you open the TWAIN interface, the **Default** Profile is selected. When you make changes to any of the live image/image capture settings, these changes can be saved to a Profile – either the Default or one that you have defined. For example, if you gamma adjust a live image and then decide that the result is ideal for the type of specimen you are working with, you could create a new Profile with the gamma and exposure values and name it for that type of specimen – i.e., "Diseased Cells."

When you close the **SPOT Camera** window, the program prompts you to save changes. Carefully consider the effect of your choice:

- Clicking **Yes** modifies the Profile according to the adjustments that you made. Future image captures taken with that Profile will reflect these changes. For example, if you rotated a live image, and then clicked Yes after closing the window, all future captures taken with that Profile would be rotated in the same way.
- Clicking **No** retains the original values of the Profile. This allows you to maintain a unique set of image view and capture settings that can be re-applied to future captures of similar specimens.

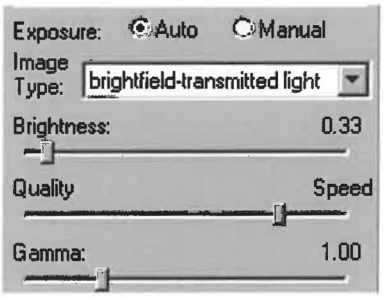
To add, edit, or delete Profiles, **right-click** on the Profile box. Right clicking gives the following options:

- **Add** – Add a new Profile. When you add a new Profile, the program prompts you to name it.
- **Save** – Save a Profile that you have modified.
- **Rename** – Rename an existing Profile.
- **Delete** - Deletea Profile. This option appears only after you have defined a second Profile in addition to the Default Profile.

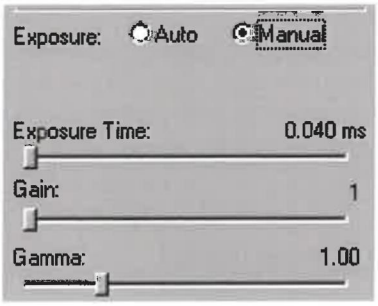
Exposure: Auto or Manual

The **Exposure** option gives you the choice of having the camera calculate exposure time (**Auto**), or setting the exposure on your own (**Manual**). The exposure options will vary according to whether you select **Auto** or **Manual**, as illustrated below.

Auto



Manual



When you enter one of the above exposure settings, note that it applies **both** to the live image that you are viewing and the captured image.

Auto Exposure options

Image Type

An Image Type tells the SPOT program how to expose and process image captures. Each of the 11 available Image Types (see below) corresponds to a microscopy technique: select the Image Type that matches the technique you are using. The camera will capture the image accordingly. The table below describes typical situations for each image type.

Image Type	Description
Brightfield-transmitted light	Stained specimens on glass slides using transmitted light microscopes.
Darkfield-transmitted light	Bright specimens on dark backgrounds using transmitted light darkfield technique.
Fluorescence	Fluorescence specimens not fluorescing in a single red, green, or blue primary color
Nomarksi DIC/Hoffman	Specimens being viewed with either Nomarski DIC or Hoffman techniques.
Phase contrast	Specimens being viewed using the phase contrast technique.
Polarized light	Specimens being viewed with polarized light techniques.
Brightfield-reflected light	Polished metallurgical specimens and semi-conductors using a microscope with a vertical illuminator.
Darkfield-reflected light	Bright areas of specimens being viewed with darkfield, reflected light techniques.

(cont.)

Image Type	Description
Stereo/macro–no glints	Matte specimens without bright glints.
Stereo/macro–shiny glints	Shiny specimens with bright glints.
Stereo/macro-metallic glints	Metallic specimens with intense glints.

Brightness

Enter a **Brightness** value between **.25** and **32**.
A brightness value of 1, the default value, gives you the correct image brightness for most images. The Brightness control allows you to overexpose or underexpose (darken or lighten) the image area. In many cases, an image has varying levels of brightness; to see a specific area better you might want to lighten or darken the image. When using the Brightness control, the SPOT camera uses an algorithm that combines gain and exposure time to provide the fastest possible image update rate (shutter speed) in live mode:

- When overexposing images to make them brighter (i.e., entering values greater than one), the camera first selects the highest possible gain before lengthening the exposure time. Because gain also brightens an image, less exposure time is needed.
- When underexposing images to make them darker (i.e., entering values less then one) the camera uses the lowest possible exposure time before lowering the gain.

Quality/Speed (Auto)

Use the slider to balance speed and quality when previewing dim images.

- Moving the slider toward the **Quality** side increases image quality and decreases frame rates.
- Moving the slider toward the **Speed** side increases frame rates and decreases image quality.

The Quality/Speed measurement corresponds to the Auto-Gain limit setting, which allows you to set an upper limit on the gain options that will be available during the Auto-Exposure process.

- When the slider is at the left-most point, the Auto-Gain Limit is 1, which restricts the camera to a gain of 1, giving you higher quality pictures, but lengthier exposure times.
- When the slider is at the right-most point, the Auto-Gain Limit is 32, which allows the camera to select from the all six gain options. This shortens exposure times, but decreases image quality.

Refer to the "Auto-Gain Limit" section in Ch. 5 – Preparing to Take a Picture for a detailed explanation of gain and exposure.

Gamma

Enter a **Gamma** value between **.1** and **4**.
Gamma adjustment smoothly lightens darker areas of an image without burning out bright areas or lightening black areas. This is especially useful for capturing/editing images with a wide range of brightness values (dynamic range) such as fluorescence specimens or macroscopic objects with glints, (e.g., printed circuitry).

- Values between **1** and **4** lighten the darker areas of the image.
- Values between **.1** and **1** darken the mid-tones of the image.

Gamma adjustments on live images are made in the RGB color space, the only color space where adjustments can be made quickly enough to keep up with live imaging. Refer to Ch. 5 – Preparing to Take a Picture for more details on gamma adjustment and color spaces.

Manual Exposure Options

Exposure Time

Use the slider-bar to specify an exposure time between **.04 ms** and **526.746 sec**.
When you specify an exposure time, the camera skips the automated exposure sequence that takes place when you open the live image view – i.e., the TWAIN interface. This is very useful in cases where exposure times are lengthy, as with fluorescence samples.

Gain

Enter the precise gain that the camera will use for an image capture. Use the slider-bar to select one of the six gain options: **1**, **2**, **4**, **8**, **16**, or **32**. The gain value displays to the right of the slider-bar. Refer to the "Auto-Gain Limit " and "Gain" sections of Ch. 5 – Preparing to Take a Picture for details on gain and exposure.

Gamma

The manual Gamma adjustment is identical to the auto Gamma adjustment (see above.)

White Balance

White balance is the ratio of red, green, and blue gains necessary to achieve the proper color rendition for an image. Many factors impact the coloration of an image capture, including lamp voltage, the coloration of the glass used in the slide, and the coloration of the glass used in the lenses of the microscope's objectives. To minimize the impact of these factors, use the White Balance feature. When you perform a white balance calculation, SPOT samples the color of the light from your light source after it has passed through the slide and the objective, and then calculates the red, green, and blue gains needed to produce images where the white areas are white and the color areas are true.

You should perform a new white balance calculation when:

- Starting a new image capture session.
- Changing the lamp voltage.
- Switching to a different objective on the microscope.

To perform a white balance, follow these steps:

1. Set the illumination and the objective that you will use for the image capture.
2. If you have not done so, select your **Image Type** (see above). Each Image Type has its own set of white balance values. Any white balance values that you compute for an image type remain linked to that image type until you compute new values. When selecting a new image type, remember that you will be using the white balance values linked to that image type.
3. Click on the **White Balance** button.

4. Show the camera a sample of white light. This step varies according to microscopy technique and Image Type (sample), as shown in the table below:

Image Type	White Balance Technique
Brightfield-transmitted light	Position the slide so that the specimen is not in the field of view, but light is going through the slide near the specimen.
Darkfield-transmitted light	Perform the white balance using the brightfield mode on your microscope. See Brightfield – transmitted light above.
Fluorescence	Use the Match Color feature (see above)
Nomarksi DIC/Hoffman	**For Nomarski DIC:** Adjust the Nomarski prism until the image is in either the "brightfield" or the "gray scale" view. **For Hoffman:** Perform the white balance directly on the Hoffman image.
Phase contrast	Position the slide so that the specimen is not in the field of view, but light is going through the slide near the specimen.
Polarized light	Pull all polarizers and compensators out of the light path and then position the slide so that the specimen is not in the field of view, but light is going through the slide near the specimen.
Brightfield-reflected light	Replace your sample with a mirror.
Darkfield-reflected light	Perform the white balance using the brightfield mode on your microscope. See Brightfield – reflected light above.
Stereo (all three options)	Using an EPI-illuminator (shining a light on the top of your specimen from off to one side), replace your sample with a white sheet of paper.

5. Click on **Begin** to start the calculation. The camera computes the white balance values, and they are automatically saved to the image settings for the image type that you selected.

Match Color

The Match Color feature is typically used in two situations:

- You want to change the colors of an image on the screen to match the way that you think the specimen looks.
- You want to set the white balance for pictures taken with a microscopic technique that never has white in its images. A prime example of this situation is fluorescence microscopy.

To use the Match Color feature, follow these steps:

1. Click on the **Preview** button to capture an image. The Match Color option remains inactive until a preview image is captured.
2. From the Edit menu, select **Match Color.** The cursor becomes an eye-dropper.
3. Using the mouse to move the eye-dropper, position it on the pixel location of the color that you want to match. The R, G, and B values of the pixel that you select must all be less than 255.
4. Click on the pixel. The **Match Color** dialog appears with information about the pixel that you selected:
   - **Hue** – The actual color, as distinguished from others in the color spectrum. Hue is derived from a color wheel and is expressed in the color's angular location (e.g., 0 to 360 degrees).
   - **Saturation** - The proportion of perceived pure hue in the color. Saturation (in the SPOT software) is measured on a numerical scale of 0 to 100, where zero equals pure gray and 100 equals pure hue (e.g., "day glow").

5. Move the cross-hairs to the desired color either by clicking on the new location or clicking and dragging. As you move the cross-hairs, the color in the image changes accordingly, allowing you to preview the color before you save the image. Alternatively, you can click on the **Make White/Grey** button to automatically white balance the image.

6. Click on **Ok**.

Chip Imaging Area

SPOT allows you to use any rectangular portion of the CCD chip for image capture. Using a portion of the chip is helpful in cases where you want to:

- Keep file sizes small.
- Shorten download times.
- Avoid areas of the image with optical problems (e.g., vignetting).

There are three image area options:

- **Full Chip** – Capture the entire area of the CCD chip.
- **Center** – Capture a rectangular part of the CCD chip's area that has the same height - to - width ratio as the full chip, and is centered on the center of the full chip. The percentage that you enter is the percentage of full chip width that the new area's width will be.

  To capture a centered proportion of an image, follow these steps:
  a) Check the Center option.
  b) Enter the percentage of the image that you want to capture. The minimum percentage is 10 and the maximum is 100.
  c) Capture the image.
- **Selection** –Capture a specific part of the CCD chip.
  a) Check the **Selection** option.
  b) Use the mouse to select a region for capture. Click and drag to define the selection. Note that the new pixel dimensions display in the status bar.
  c) Capture the image.

When you are viewing a live image **Selection**, how this selection is displayed, depends on two other settings: the **Fit to Window** option (see below) and the **Always Show Full Chip in Live Mode** preference (see above). The following table illustrates the possibilities:

Fit to Window Checked?	Always Show Full Chip in Live Mode Checked?	Selection Display	Live or Captured Image
YES	YES	When you select a portion of the chip, the selection is outlined with a **yellow rectangle**.	LIVE
YES	NO	When you select a portion of the chip the cursor is a **zoom tool**. To zoom in on a portion of a live or captured image, click and drag over the area that you want to zoom in on. To return to the non-zoomed image, click on the **Full Chip** option.	BOTH
NO	YES	When you select a portion of the chip, the selection is outlined with a yellow rectangle. However, because **Fit to Window** is not checked, you will not see the entire chip in the window.	LIVE
NO	NO	When you select a portion of the image, the cursor is a cropping tool. If you select a portion of an image, the program displays only the selected area and discards the rest of the image. To return to the non-cropped image, click on the	BOTH

Full Chip option.

Auto-Brightness (Auto, Live Images)

Available only when you are using **Auto** exposure and viewing a live image, the **Auto-Brightness** option enables you to maintain a consistent brightness level for changing specimens.

Faster Readout (Live Images)

Faster Readout enables you to "speed up" the live image display (higher frame rates) in cases where you are willing to sacrifice some resolution for a higher frame rate.

Flatfield (Captured Images)

For all other SPOT camera lines, the Flatfield Correct option is used to correct for display problems associated with uneven intensity or coloration in illumination, or to correct for artifacts (e.g., dust) in the optical system. Flatfield can still be used for this purpose with SPOT Insight cameras. But, because SPOT Insight cameras use a color mosaic chip, Flatfield correction serves a new primary purpose. In the production process for color mosaic CCD chips, the RGB colors are applied directly to the pixels on the chip. This process often results in an uneven color density around the outer 30-40 rows of the CCD chip, causing a "halo" appearance in some image captures, particularly those with low contrast. The Flatfield option can be used to correct this problem.

Note: Flatfield correction with the SPOT camera refers only to the correction of uneven lighting. It should not be confused with the correction of optical field flatness.

Flatfield correction works by taking a picture of the brightfield background without the specimen in the field of view. This image lets the SPOT software know, on a pixel-by pixel basis, which areas in the field of view are receiving less illumination than the brightest areas. When taking a picture that contains a specimen, the software uses this information to accurately increase the brightness of each pixel in the dimly illuminated areas. The resulting image gives the appearance of an even illumination.

To use the **Flatfield** option, follow these steps:

1. Ensure that the specimen is under the desired objective and in focus.
2. Right click on the **Flatfield** drop-down list and click on the **Add** option. The **Get Flatfield** dialog appears as shown at right.
3. Remove the slide from the field of view.
4. Click on Begin. When the program finishes computing the exposure times and acquiring the flatfield file, the **Flatfield** dialog prompts you to name the file:
   a) Enter a name. Because flatfield image files are different for each objective, you might want to name the file according to the objective's magnification (e.g., 10x).
   b) Click on OK. If you do not specify a location, the program automatically saves the flatfield file to the **c:\SpotCam\Fltflds** directory.

Note: The default file location varies, depending on where you installed the SPOT program.

5. Move the specimen back into the field of view, and perform the image capture, using either the **Preview** or the **Acquire** option.

Color Enhancement (Captured Images)

The Color Enhancement option optimizes image captures by closely matching the final capture to the image as seen through the microscope. To optimize the final image, the SPOT program processes the raw image data to produce a final image that matches the view seen through the microscope.

If you want an image capture to match the live image view, you will want to leave Color Enhancement off. Color Enhancement applies only to captured images.

Fit to Window

In addition to automatically resizing images to fit within the live image window, the Fit to Window option also determines cursor behavior in the live image view.

- When the **Fit to Window** option is checked, the live image window **cursor** becomes a **zoom tool**. To zoom in on a portion of a live image, click and drag over the area that you want to zoom in on. To return to the non-zoomed image, click on the **Full Chip** option.
- When the **Fit to Window** option is not checked, the cursor is a cropping tool. If you select a portion of an image, the program displays only the selected area and discards the rest of the image. To return to the non-cropped image, click on the **Full Chip** option.

These points apply *only* when the **Always Show Full Chip in Live Mode** (see above) is *not selected.* For more details, refer back to the **Chip Imaging Area** section (see above).

Note: Due to a flaw in the Windows operating system, monitors that are set to higher resolutions cannot zoom to very high magnification levels. If zoomed in to a high magnification, the monitor displays white instead of the zoomed image.

More (Additional Orientation and Exposure Settings)

When you click on **More**, the program displays a dialog of additional orientation and exposure settings for the TWAIN interface, as shown at right.

Flip

Specify whether images will be flipped in the live image view and during the image capture process.

- Select **Horizontal** to flip the image from left to right.
- Select **Vertical** to flip the image from top to bottom.
- Select both options to flip the image from left to right and from top to bottom.

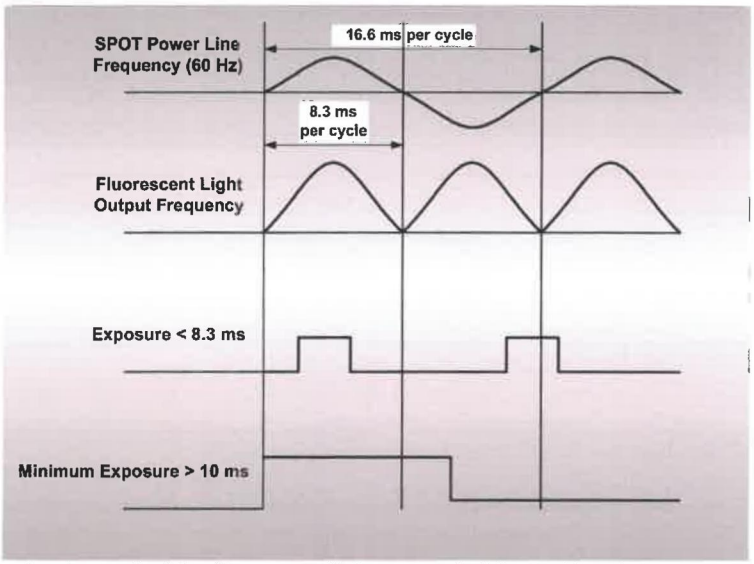
Rotate 90°

Specify whether images will be rotated in the live image view and during the image capture process.

- Select **None** if you do not want the image to be rotated.
- Select **Right** to rotate the image clockwise 90 degrees.

- Select **Left** to rotate the image counterclockwise 90 degrees.

Exposure Limits – Minimum



Setting a Minimum Exposure to Accommodate Varied Light Sources

Because SPOT Insight cameras are capable of extremely short exposure times, light sources with intensities that vary with the waveform of the 60 Hz line supply (e.g., fluorescent lamps, 120 volt incandescent bulbs, and 6 volt 20 watt halogen lamps) can cause the Live Image window to flicker and result in incorrect brightness and color balance readings in captured images. This option allows you to correct for these problems by limiting the camera to a minimum exposure time that is greater than the pulse interval of the light source. Consider the illustration to the right. In this case, the pulse interval of the fluorescent light source is 8.3 ms. Entering a Minimum Exposure of 10 ms ensures that the camera's exposure time is greater than the pulse interval of the light source. To enter a minimum exposure, check the **Minimum** box, and enter a value in milliseconds.

Exposure Limits – Live Image Maximum

Because The Live Image Maximum option helps you to avoid unnecessarily long exposures when viewing a live image. Consider the following situation: When you change from a lower resolution objective to a higher resolution objective, the camera detects a brief interval of blackness. When the Auto-Brightness option (see above) is enabled, it compensates by modifying the exposure time to maintain a constant brightness level. This results in you having to wait for the camera to compute a lengthy and incorrect exposure for the black interval. Using the **Live Image Maximum** option, you can limit this exposure time. For most cases, Diagnostic Instruments recommends a value of 0.5 seconds.

Similarly, the Live Image Maximum option can also be used in cases where the microscope's light path selector is incorrectly set.

Noise Filter

The Noise Filter corrects for the effects of electrical or thermal noise. When capturing dim images with lengthy exposure times, the noise filter acts as a "virtual cooler" for the Insight camera, eliminating "hot pixels."

The Noise Filter checks each pixel on the CCD chip as described below:

1. The program compares the value of each red pixel with the values of the eight neighboring red pixels.
2. If none of the eight neighboring red pixels has a value within ±15 % (i.e., the threshold value that the SPOT program uses) of the value for the red pixel being checked, the value for the red pixel being checked is replaced with the average value of the eight neighboring red pixels.

3. The program repeats this procedure for the values of the green and blue pixels being checked.

To activate the Noise Filter, check the Noise Filter box. The program runs the noise filter each time an image is captured using the **Preview** or **Acquire** options.

Taking a Picture

From the SPOT TWAIN Interface, you can capture images in different ways:

- **Preview** - You can capture a **Preview** image. Preview images remain available in the TWAIN window for basic editing and serve as the basis for the final image capture. When you click on the **Preview** button, the **Get Image** dialog displays the capture status as it is captured, and the captured image then replaces in the Live Image in the window. Note that the status bar indicated that you are viewing a Preview image.
- **Transfer** - If you like the Preview image, you can **Transfer** it image directly to the third party application that you are using. The Transfer button is active only for Preview image captures.
- **Acquire** - Capture an image and have it automatically transfer to (open in) the third party imaging application.

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QUICK REFERENCE:

- Define file and save options for the 3rd Party Interface... p. 230
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Introduction

The SPOT 3rd Party Interface is a stand-alone mini-application that is designed for Mac users who want to use the SPOT camera to capture images, and then edit the images in a third party imaging application (e.g., Photoshop, Image-Pro). Using the 3rd Party Interface, you can:

- View a live image preview
- Create and/or modify a SPOT image setup
- Capture an image and have it automatically open in another application
- Capture an image and save it to file (i.e., the image is not associated with a specific application)
- Transfer captured images into the image editing software

You *cannot*, however, use the 3rd Party Interface to open images from file.

Preparing to Take a Picture

Before taking a picture via the 3rd Party Interface, you can set the following parameters as needed:

- The SPOT 3rd Party Interface File and Save Options
- The SPOT 3rd Party Interface live image viewing preferences
- The Image Setup, which defines how the camera will take pictures

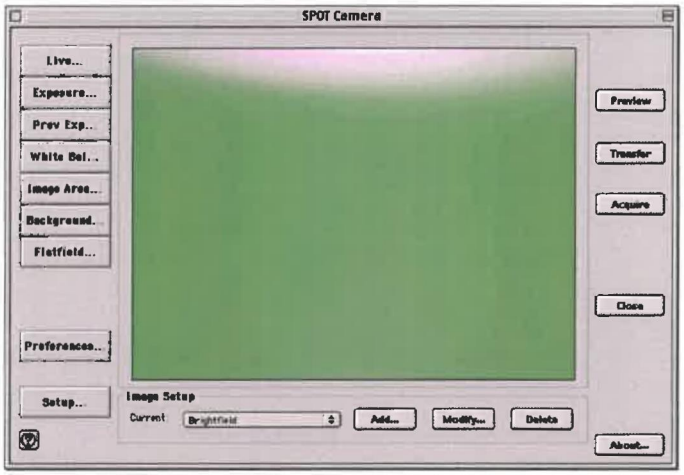
Setting File and Save Options

Before using the 3rd Party Interface program, you can specify file format, open, and save options. This includes the following information:

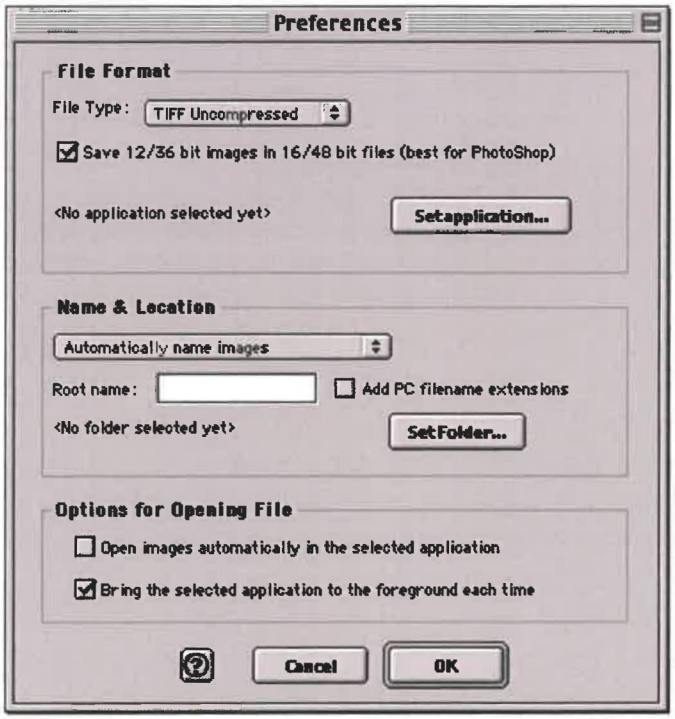
- File format for captured images
- Imaging application to edit captured images
- Location for saved image files
- File naming conventions
- File opening conventions

To specify this information, follow these steps:

1. Open the 3rd Party Interface. The **SPOT Camera** window appears, as shown at right.



2. Click on the **Setup** button. The **Preferences** window appears.



3. Specify the **File Type**. Click on the File Type selector and select one of the following options:
   - Windows Bitmap
   - Mac PICT (pct, pic, pict)
   - TIFF Uncompressed (tif, tiff)

> *Note: To capture images at pixel bit depth settings other than 8 or 24 bpp (e.g., 12 or 36 bpp), you should select the* **TIFF/Uncompressed** *file type.*

4. If you use the 12 bpp or 36 bpp setitngs, and you use an imaging application that saves files in 16 bpp and 48 bpp format (e.g., Photoshop), ensure that the **Save 12/36 bit images in 16/48 bit files (best for Photoshop)** box is checked.

5. Click on the **Set Application** button to specify the imaging application (e.g., Photoshop) that you will use with the SPOT camera and the 3rd Party Interface.

6. Specify how files are named and where they are saved to. There are two options.
   - **Automatically name images** – The program automatically names and saves images according to the parameters that you specify.
   - **Ask for filename each time** - Have the program prompt you to enter the name and file location each time.

   If you choose the **Automatically name images** option, specify the **Root name** for the image files and the folder that they will be saved to.
   - Enter a name in the **Root name** box. The Root name is the first part of the file name. If the root name is **test**, for example, the first image that you save will be **test0001**, the second image **test0002**, etc… If you do not specify a Root name, the files are saved with the numerical names (e.g., 0001, 0002, etc…).
   - To display the PC file type extension for image files, check the **Add PC filename extensions** box.
   - Click on the **Set Folder** button to specify where you will save the file. If you do not specify a location, the 3rd Party Interface program creates a folder on the desktop called SPOT Images; captured images are automatically saved to this location.

7. Specify the file open options. You can select one, both, or neither of the two options:
   - **Open images automatically in selected application** – When you Acquire or Transfer images, the images open automatically in the application that you specified in step 4 above.
   - **Bring the selected image to the foreground each time** – When you Acquire or Transfer images, the imaging application window opens in front of the SPOT Camera window, which is sent to the background.

   If neither option is selected, the image file is saved to the location that you specify.

8. Click on OK. The file type and application that you specified appear next to the Change button on the SPOT Camera window.

Defining Live Image Preferences

The 3rd Party Interface window includes a **Preferences** button, which allows you to define live image viewing and exposure preferences. This includes the following options:

- Always Show Full Chip
- Maximum Exposure
- Do Post Processing On

Each option is discussed below.

Always Show Full Chip

Always display the full chip in live image mode. If you select a region, it will be displayed in a rectangular outline within the full-chip view.

Maximum Exposure

The Maximum Exposure option helps you to avoid unnecessarily long exposures when viewing a live image. Consider the following situation: When you change from a lower resolution objective to a higher resolution objective, the camera detects a brief interval of blackness and compensates by modifying the exposure time to maintain a constant brightness level. This results in you having to wait for the camera to

compute a lengthy and incorrect exposure for the black interval. Using the **Maximum Exposure** option, you can limit this exposure time. For most cases, Diagnostic Instruments recommends a value of 0.5 seconds. Similarly, the Live Image Maximum option can also be used in cases where the microscope's light path selector is incorrectly set.

Post Processing

Specify whether to apply the Post Processing options for the current Image Setup to images captured from the live image view.

Transferred Images

- *If the Transferred Images box is selected,* the Transfer option applies the Noise Filter, Color Enhancement, and Image Size Double options as specified for the current Image Setup.
- *If the Transferred Images box is unselected,* the Transfer option ignores the Post Processing options for the current Image Setup, with one exception: if the Chip Defect Correction option is selected for the current image setup (SPOT and RT cameras only) , the corrections will appear in the image capture, regardless of the Transferred Images preference.

Snapped Images

- *If the Snapped Images box is selected,* the Snap option uses all of the Post Processing options from the current Image Setup, except for Background Subtraction and Flatfield Correction.
- *If the Snapped Images box is unselected,* the Snap option ignores the Post Processing options for the current Image Setup, with one exception: if the Chip Defect Correction option is selected for the current image setup (RT and SPOT cameras only), the corrections will appear in the image capture, regardless of the Snapped Images preference.

Palettes (Insight B/W)

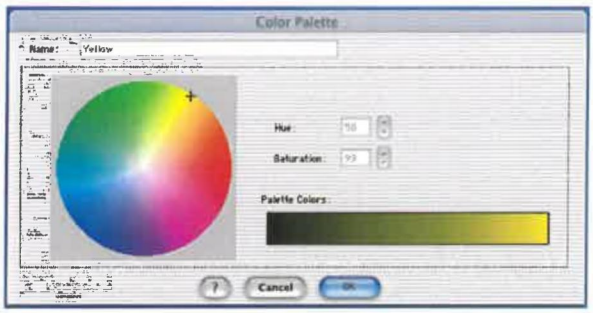
The Palettes option allows you to define new palettes and edit existing palettes.

SPOT color palettes allow you to apply specific hue and saturation values to monochrome (8 bit or 12 bit) images. Palettes are useful in fluorescence applications where a sample is fluorescing at a single wavelength, and you want the acquired image to have the correct color. For such a case, you could create a palette to represent the color of the fluorescent dye and then apply that palette to the captured image. There are two ways to apply a palette to an image:

- You can associate a palette with an Image Setup, as discussed in Ch. 5 - Preparing to Take a Picture.
- You can apply a palette to a captured image by selecting the **Set Palette** option (Advanced mode) from the **Edit** menu. Refer to Ch. 8 – Viewing, Editing, and Annotating Images for more details.

To define a new palette, follow these steps:

1. From the **Setup** menu, select **Palettes.**
2. Click on **Add**. The Color Palette dialog appears as shown here:



3. Either use the **Hue** and **Saturation** selection lists or move the cross-hairs on the color wheel to enter the values
   - **Hue** is the designation for the actual color, as distinguished from others in the color spectrum. Hue is derived from the color wheel and is expressed in the color's angular location (in degrees) on the wheel. (e.g., 0° to 360°).
   - **Saturation** is the proportion of perceived pure hue in the color. Saturation (in the SPOT software) is measured on a numerical scale of 0 to 100, where zero is pure gray and 100 is pure hue (e.g., day glow).
4. Click on **OK**.

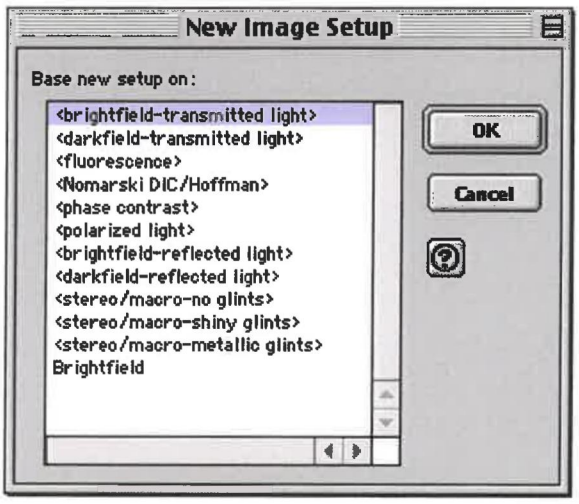
Defining an Image Setup

An image setup serves two crucial purposes:

- It defines the way a picture will be taken.
- It defines the way the program automatically modifies a picture after it is taken.

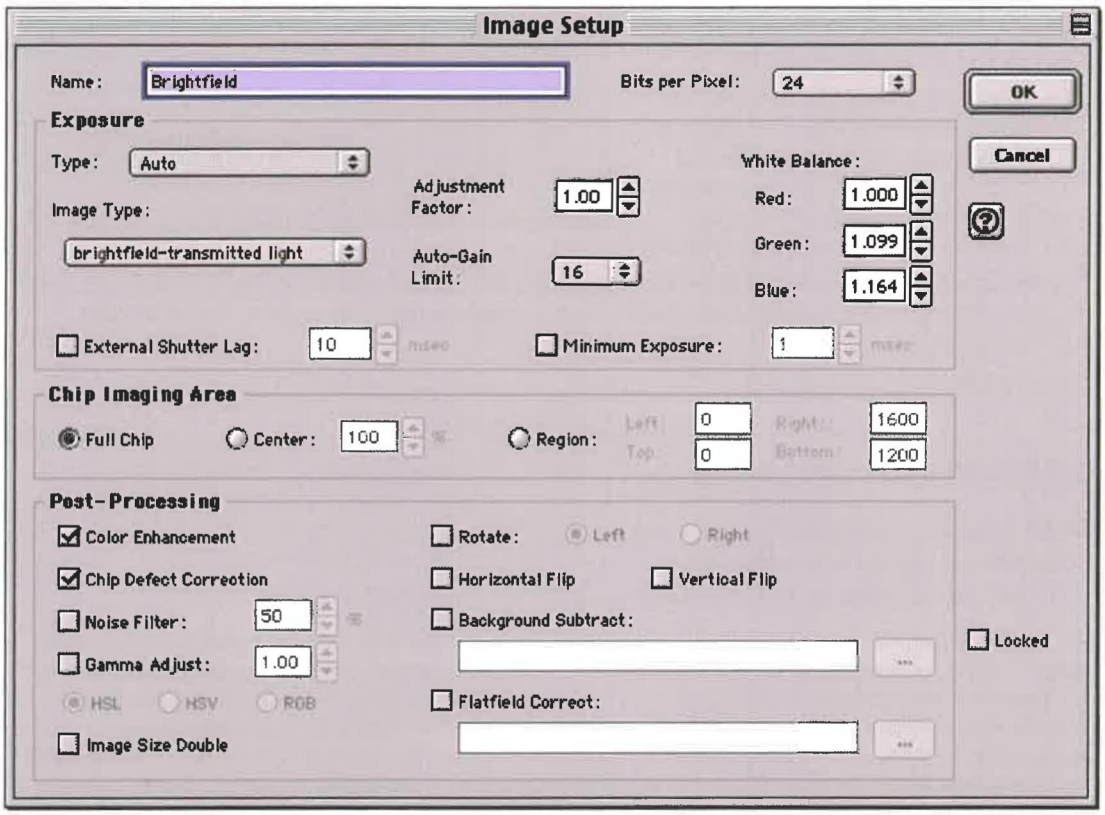
Image setups are defined to optimize the picture taking process for different types of images or microscopy techniques. They can be created, saved, and recalled as needed. To define a new image setup, you must also select one of 14 pre-defined image types as a template for defining the new setup. The 14 pre-defined image types (in addition to the Factory Defaults setting) correspond to commonly used microscopy techniques. Refer to Ch. 5- Preparing to Take a Picture for a table of the different image types. Pre-defined image types are indicated by brackets around the title, while the user defined image setups appear without brackets.

- To modify the current image setup, click on the **Modify** button.
- To delete an image setup, select the image setup from the list, and click on the **Delete** button.
- To define a new image setup, click on the **Add** button. The New Image Setup window appears with a list of pre-defined and user-defined templates (image types). Pre-defined image types are indicated by brackets < >. Select a template, and click on OK. The New Image Setup window appears, as shown at right:



Insight B/W	Insight B/W camera users will not see the fluorescence – red, fluorescence – green, and fluorescence – blue Image Type options.

When you add a new image setup or modify an existing setup, the Image Setup window appears, as shown here.



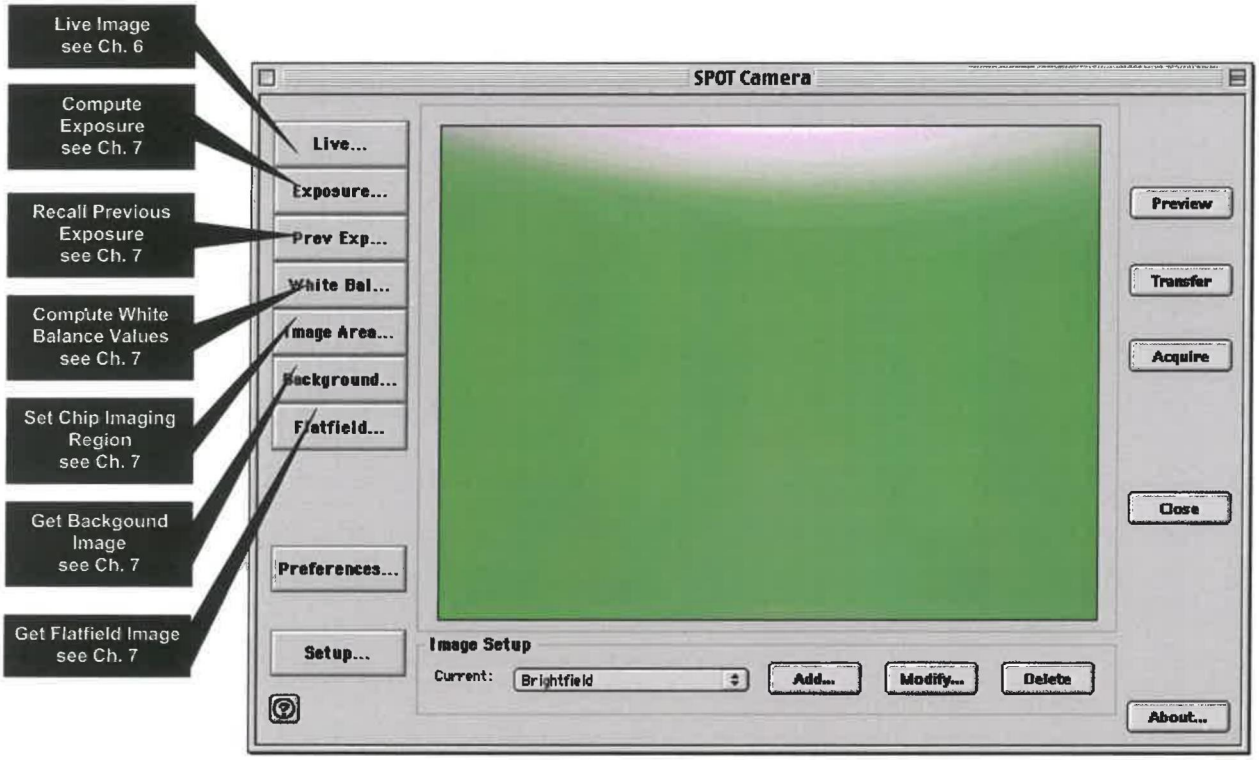
Refer to Ch. 5 – Preparing to Take a Picture for a detailed explanation of the Image Setup options.

Taking a Picture

Using the 3rd Party Interface, you can perform both initial image captures (e.g., for computing exposure, white balance, etc…) and final image captures.

Capturing Initial Images

The toolbar at the left of the SPOT Image Capture window enables you to quickly access the image capture functions that are used prior to the capture of the final image. The call-outs below page illustrate the toolbar buttons:



Insight B/W	Insight B/W camera users will not see the Compute White Balance Values toolbar button.

Capturing Final Images

From the 3rd Party Interface program, you can capture an image in two different ways. You can either preview the image and then transfer it to another imaging application (e.g., Photoshop), or you can Acquire the image, which sends the captured image directly to the application.

- **Preview** – Capture an image and preview it within the SPOT Image Capture window.
- **Transfer** – Transfer a captured image to the imaging application.
- **Acquire** - Capture an image and have it open/save according to the parameters that you specified (see Setting File and Save Options). The one-step process of Acquiring an image is identical to previewing and then transferring an image

Ch. 15 – Using Other Mac Imaging Applications in Basic Mode

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- Perform a white balance on an image... p. 244
- View or capture a region of an image... p. 246
- Capture an image... p. 250

Introduction

The SPOT Basic 3rd Party Interface is a stand-alone mini-application that is designed for Mac users who want to use the SPOT camera to capture images, and then edit the images in a third party imaging application (e.g., Photoshop, Image-Pro). Using the 3rd Party Interface, you can:

- View a live image preview
- Create and/or modify a SPOT image setup
- Capture an image and have it automatically open in another application
- Capture an image and save it to file (i.e., the image is not associated with a specific application)
- Transfer captured images into the image editing software

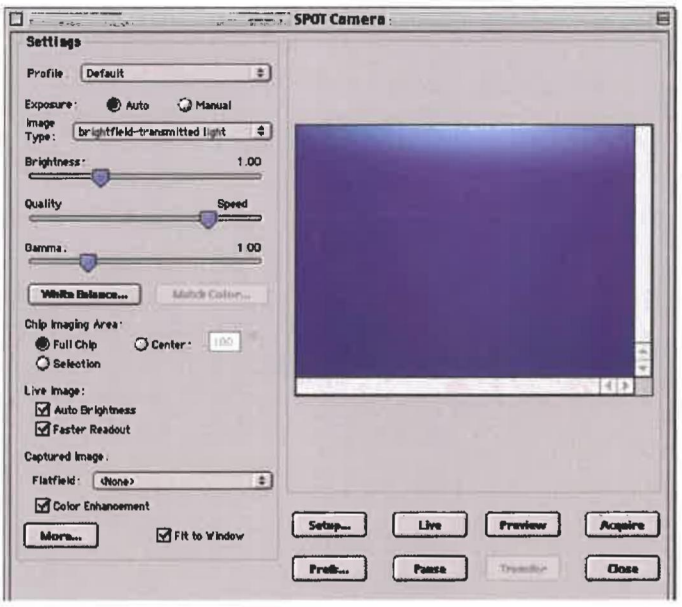
You *cannot*, however, use the 3rd Party Interface to open images from file.

Working in the Basic 3rd Party Interface

The Basic 3rd Party interface is a one-stop point for previewing and capturing images. For example, using the Basic 3rd Party interface, a typical image capture session might involve the following steps, as illustrated below:

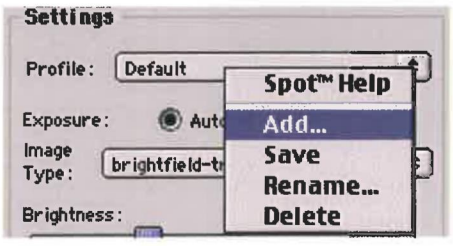
Preview your specimen

Use the live image as a point of reference to focus and position the image and to scan for areas of interest.



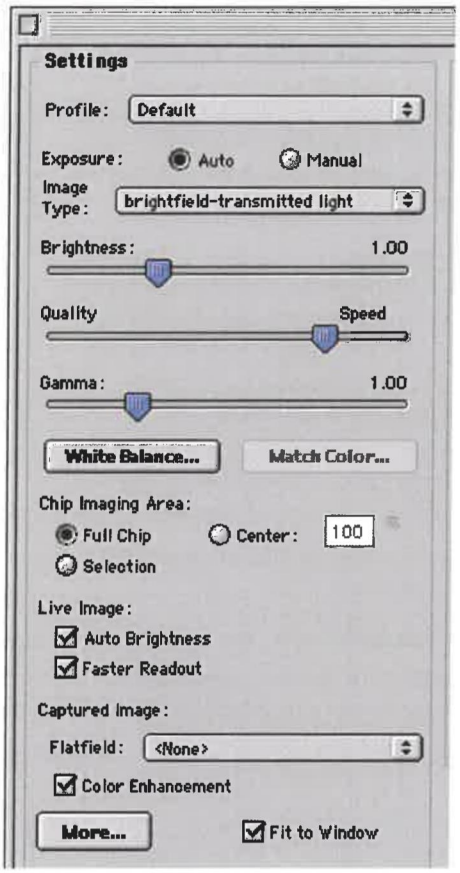
Create a Profile

Profiles allow you to create, modify, and reuse specific exposures settings that are tailored to your image capture needs. To create a new profile, **control-click** on the Profile box.



Define Settings

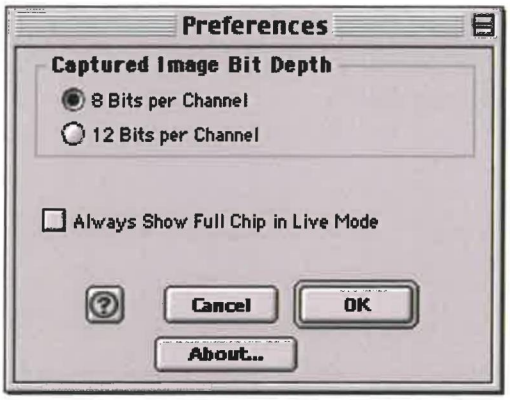
Define exposure settings for live and captured images. Each of these settings are discussed later in this chapter.



Capture an Image

All of the image capture options are located on the bottom portion of the window, as illustrated here

Defining Preferences



In the Basic 3rd Party Interface, you can define three capture/view preferences. Click on the **Prefs** button to open the **Preferences** window (see right). Each option is described below:

Captured Image Bit Depth
This option enables you to specify a different image bit depth when saving an image captured in the SPOT program.

- **8 Bits per Channel** – The 8 bits per channel option provides 256 gray levels per color:
  - With Insight B/W cameras, captured images are 8 bits per pixel monochrome.
  - With Insight Color cameras, captured images are 24 bits per pixel RGB color.

  This option results in smaller, more manageable image sizes, and is best for images that do not require extensive modification.

- **12 Bits per Channel** – The 12 Bits per channel option provides 4,096 gray levels per color.
  - With Insight B/W cameras, captured images are 12 bits per pixel monochrome.
  - With Insight Color cameras, captured images are 36 bits per pixel RGB color.

  This option reduces banding problems that can result with modified images, but also results in larger file sizes.

  When saving 12 bit per channel images, SPOT prompts you to select either 12 bits per channel or 16 bits per channel. If you use Adobe's Photoshop for image editing, you should select the 16 bit per channel option because Photoshop cannot read 12 bit per channel images.

Note: The SPOT Insight camera captures all images using 10 bits per channel (color). The SPOT software immediately converts the captured image to either 8 bits per channel (color) or 12 bits per channel (color), depending on the **Captured Image Bit Depth** *that you select.*

Always Show Full Chip in Live Mode
Checking this option ensures that you will always see the Full Chip view when viewing a live image. Keep in mind that the selection that you make here determines how the live image displays when viewing a region of the chip. Refer to the **Chip Imaging Area** section for more details.

F-Keys

The following function keys are available as shortcuts in the Basic 3rd Party interface:

Function Key	Action	3rd Party Interface Option
[F 4]	Restart the live image view.	Live
[Alt] + [F 4]	Close the SPOT Camera window.	Close
[F 9]	Capture an image and automatically transfer it to the imaging application.	Acquire
[Ctrl] + [F 10]	Compute white balance values.	White Balance
[Alt] + [F 10]	Compute white balance values without displaying the informational dialog.	*Not available from the window*

Using the Live Image to Define Image Capture Settings

When you open the Basic mode 3rd Party Interface, the Live Image window is, by default, running, as indicated by the **Live Mode Running** message in the status bar. The settings options in the 3rd Party interface window enable you to optimize the live image view, and then capture an image using these settings. In the 3rd Party Interface, you can:

- Create profiles to use as pre-defined image capture settings.
- Modify exposure settings
- Define a specific portion of the chip to be used in the image capture.
- Modify the screen orientation of the specimen.

The following sections describe all of the Live Image/Image Capture Settings.

Profile

The **Profile** option allows you to create and re-use sets of live image/image capture settings. When you open the 3rd Party interface, the **Default** Profile is selected. When you make changes to any of the live image/image capture settings, these changes can be saved to a Profile – either the Default or one that you have defined. For example, if you gamma adjust a live image and then decide that the result is ideal for the type of specimen you are working with, you could create a new Profile with the gamma and exposure values and name it for that type of specimen – i.e., "Diseased Cells."

When you close the **SPOT Camera** window, the program prompts you to save changes. Carefully consider the effect of your choice:

- Clicking **Yes** modifies the Profile according to the adjustments that you made. Future image captures taken with that Profile will reflect these changes. For example, if you rotated a live image, and then clicked Yes after closing the window, all future captures taken with that Profile would be rotated in the same way.
- Clicking **No** retains the original values of the Profile. This allows you to maintain a unique set of image view and capture settings that can be re-applied to future captures of similar specimens.

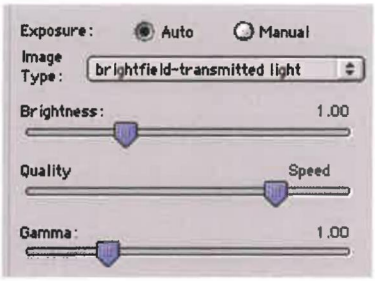
To add, edit, or delete Profiles, **right-click** on the Profile box. Right clicking gives the following options:

- **Add** – Add a new Profile. When you add a new Profile, the program prompts you to name it.
- **Save** – Save a Profile that you have modified.
- **Rename** – Rename an existing Profile.
- **Delete** - Deletea Profile. This option appears only after you have defined a second Profile in addition to the Default Profile.

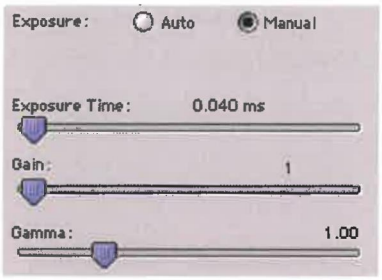
Exposure: Auto or Manual

The **Exposure** option gives you the choice of having the camera calculate exposure time (**Auto**), or setting the exposure on your own (**Manual**). The exposure options will vary according to whether you select **Auto** or **Manual**, as illustrated below.

Auto



Manual



When you enter one of the above exposure settings, note that it applies **both** to the live image that you are viewing and the captured image.

Auto Exposure options

Image Type

An Image Type tells the SPOT program how to expose and process image captures. Each of the 11 available Image Types (see below) corresponds to a microscopy technique: select the Image Type that matches the technique you are using. The camera will capture the image accordingly. The table below describes typical situations for each image type.

Image Type	Description
Brightfield-transmitted light	Stained specimens on glass slides using transmitted light microscopes.
Darkfield-transmitted light	Bright specimens on dark backgrounds using transmitted light darkfield technique.
Fluorescence	Fluorescence specimens not fluorescing in a single red, green, or blue primary color
Nomarksi DIC/Hoffman	Specimens being viewed with either Nomarski DIC or Hoffman techniques.
Phase contrast	Specimens being viewed using the phase contrast technique.
Polarized light	Specimens being viewed with polarized light techniques.
Brightfield-reflected light	Polished metallurgical specimens and semi-conductors using a microscope with a vertical illuminator.
Darkfield-reflected light	Bright areas of specimens being viewed with darkfield, reflected light techniques.

(cont.)

Image Type	Description
Stereo/macro–no glints	Matte specimens without bright glints.
Stereo/macro–shiny glints	Shiny specimens with bright glints.
Stereo/macro-metallic glints	Metallic specimens with intense glints.

Brightness

Enter a **Brightness** value between **.25** and **32**.
A brightness value of 1, the default value, gives you the correct image brightness for most images. The Brightness control allows you to overexpose or underexpose (darken or lighten) the image area. In many cases, an image has varying levels of brightness; to see a specific area better you might want to lighten or darken the image. When using the Brightness control, the SPOT camera uses an algorithm that combines gain and exposure time to provide the fastest possible image update rate (shutter speed) in live mode:

- When overexposing images to make them brighter (i.e., entering values greater than one), the camera first selects the highest possible gain before lengthening the exposure time. Because gain also brightens an image, less exposure time is needed.
- When underexposing images to make them darker (i.e., entering values less then one) the camera uses the lowest possible exposure time before lowering the gain.

Quality/Speed (Auto)

Use the slider to balance speed and quality when previewing dim images.

- Moving the slider toward the **Quality** side increases image quality and decreases frame rates.
- Moving the slider toward the **Speed** side increases frame rates and decreases image quality.

The Quality/Speed measurement corresponds to the Auto-Gain limit setting, which allows you to set an upper limit on the gain options that will be available during the Auto-Exposure process.

- When the slider is at the left-most point, the Auto-Gain Limit is 1, which restricts the camera to a gain of 1, giving you higher quality pictures, but lengthier exposure times.
- When the slider is at the right-most point, the Auto-Gain Limit is 32, which allows the camera to select from the all six gain options. This shortens exposure times, but decreases image quality.

Refer to the "Auto-Gain Limit" section in Ch. 5 – Preparing to Take a Picture for a detailed explanation of gain and exposure.

Gamma

Enter a **Gamma** value between **.1** and **4**.
Gamma adjustment smoothly lightens darker areas of an image without burning out bright areas or lightening black areas. This is especially useful for capturing/editing images with a wide range of brightness values (dynamic range) such as fluorescence specimens or macroscopic objects with glints, (e.g., printed circuitry).

- Values between **1** and **4** lighten the darker areas of the image.
- Values between **.1** and **1** darken the mid-tones of the image.

Gamma adjustments on live images are made in the RGB color space, the only color space where adjustments can be made quickly enough to keep up with live imaging. Refer to Ch. 5 – Preparing to Take a Picture for more details on gamma adjustment and color spaces.

Manual Exposure Options

Exposure Time

Use the slider-bar to specify an exposure time between **.04 ms** and **526.746 sec**.
When you specify an exposure time, the camera skips the automated exposure sequence that takes place when you open the live image view – i.e., the 3rd Party interface. This is very useful in cases where exposure times are lengthy, as with fluorescence samples.

Gain

Enter the precise gain that the camera will use for an image capture. Use the slider-bar to select one of the six gain options: **1**, **2**, **4**, **8**, **16**, or **32**. The gain value displays to the right of the slider-bar. Refer to the "Auto-Gain Limit " and "Gain" sections of Ch. 5 – Preparing to Take a Picture for details on gain and exposure.

Gamma

The manual Gamma adjustment is identical to the auto Gamma adjustment (see above.)

White Balance

White balance is the ratio of red, green, and blue gains necessary to achieve the proper color rendition for an image. Many factors impact the coloration of an image capture, including lamp voltage, the coloration of the glass used in the slide, and the coloration of the glass used in the lenses of the microscope's objectives. To minimize the impact of these factors, use the White Balance feature. When you perform a white balance calculation, SPOT samples the color of the light from your light source after it has passed through the slide and the objective, and then calculates the red, green, and blue gains needed to produce images where the white areas are white and the color areas are true.

You should perform a new white balance calculation when:

- Starting a new image capture session.
- Changing the lamp voltage.
- Switching to a different objective on the microscope.

To perform a white balance, follow these steps:

1. Set the illumination and the objective that you will use for the image capture.
2. If you have not done so, select your **Image Type** (see above). Each Image Type has its own set of white balance values. Any white balance values that you compute for an image type remain linked to that image type until you compute new values. When selecting a new image type, remember that you will be using the white balance values linked to that image type.
3. Click on the **White Balance** button.
4. Show the camera a sample of white light. This step varies according to microscopy technique and Image Type (sample), as shown in the table below:

Image Type	White Balance Technique
Brightfield-transmitted light	Position the slide so that the specimen is not in the field of view, but light is going through the slide near the specimen.
Darkfield-transmitted light	Perform the white balance using the brightfield mode on your microscope. See Brightfield – transmitted light above.
Fluorescence	Use the Match Color feature (see above)
Nomarksi DIC/Hoffman	**For Nomarski DIC:** Adjust the Nomarski prism until the image is in either the "brightfield" or the "gray scale" view. **For Hoffman:** Perform the white balance directly on the Hoffman image.
Phase contrast	Position the slide so that the specimen is not in the field of view, but light is going through the slide near the specimen.
Polarized light	Pull all polarizers and compensators out of the light path and then position the slide so that the specimen is not in the field of view, but light is going through the slide near the specimen.
Brightfield-reflected light	Replace your sample with a mirror.
Darkfield-reflected light	Perform the white balance using the brightfield mode on your microscope. See Brightfield – reflected light above.
Stereo (all three options)	Using an EPI-illuminator (shining a light on the top of your specimen from off to one side), replace your sample with a white sheet of paper.

5. Click on **Begin** to start the calculation. The camera computes the white balance values, and they are automatically saved to the image settings for the image type that you selected.

Match Color

The Match Color feature is typically used in two situations:

- You want to change the colors of an image on the screen to match the way that you think the specimen looks.
- You want to set the white balance for pictures taken with a microscopic technique that never has white in its images. A prime example of this situation is fluorescence microscopy.

To use the Match Color feature, follow these steps:

1. Click on the **Preview** button to capture an image. The Match Color option remains inactive until a preview image is captured.
2. Select **Match Color.** The cursor becomes an eye-dropper.
3. Using the mouse to move the eye-dropper, position it on the pixel location of the color that you want to match. The R, G, and B values of the pixel that you select must all be less than 255.
4. Click on the pixel. The **Match Color** dialog appears with information about the pixel that you selected:
   - **Hue** – The actual color, as distinguished from others in the color spectrum. Hue is derived from a color wheel and is expressed in the color's angular location (e.g., 0 to 360 degrees).
   - **Saturation** - The proportion of perceived pure hue in the color. Saturation (in the SPOT software) is measured on a numerical scale of 0 to 100, where zero equals pure gray and 100 equals pure hue (e.g., "day glow").
5. Move the cross-hairs to the desired color either by clicking on the new location or clicking and dragging. As you move the cross-hairs, the color in the image changes accordingly, allowing you to preview the color before you save the image. Alternatively, you can click on the **Make White/Grey** button to automatically white balance the image.
6. Click on **Ok.**

Chip Imaging Area

SPOT allows you to use any rectangular portion of the CCD chip for image capture. Using a portion of the chip is helpful in cases where you want to:

- Keep file sizes small.
- Shorten download times.
- Avoid areas of the image with optical problems (e.g., vignetting).

There are three image area options:

- **Full Chip** – Capture the entire area of the CCD chip.
- **Center** – Capture a rectangular part of the CCD chip's area that has the same height - to - width ratio as the full chip, and is centered on the center of the full chip. The percentage that you enter is the percentage of full chip width that the new area's width will be.

  To capture a centered proportion of an image, follow these steps:
  a) Check the Center option.
  b) Enter the percentage of the image that you want to capture. The minimum percentage is 10 and the maximum is 100.
  c) Capture the image.
- **Selection** –Capture a specific part of the CCD chip.
  a) Check the **Selection** option.
  b) Use the mouse to select a region for capture. Click and drag to define the selection. Note that the new pixel dimensions display in the status bar.
  c) Capture the image.

When you are viewing a live image **Selection**, how this selection is displayed, depends on two other settings: the **Fit to Window** option (see below) and the **Always Show Full Chip in Live Mode** preference (see above). The following table illustrates the possibilities:

Fit to Window Checked?	Always Show Full Chip in Live Mode Checked?	Selection Display	Live or Captured Image
YES	YES	When you select a portion of the chip, the selection is outlined with a **yellow rectangle**.	LIVE
YES	NO	When you select a portion of the chip the cursor is a **zoom tool**. To zoom in on a portion of a live or captured image, click and drag over the area that you want to zoom in on. To return to the non-zoomed image, click on the **Full Chip** option.	BOTH
NO	YES	When you select a portion of the chip, the selection is outlined with a yellow rectangle. However, because **Fit to Window** is not checked, you will not see the entire chip in the window.	LIVE
NO	NO	When you select a portion of the image, the cursor is a cropping tool. If you select a portion of an image, the program displays only the selected area and discards the rest of the image. To return to the non-cropped image, click on the **Full Chip** option.	BOTH

Auto-Brightness (Auto, Live Images)

Available only when you are using **Auto** exposure and viewing a live image, the **Auto-Brightness** option enables you to maintain a consistent brightness level for changing specimens.

Faster Readout (Live Images)

Faster Readout enables you to "speed up" the live image display (higher frame rates) in cases where you are willing to sacrifice some resolution for a higher frame rate.

Flatfield (Captured Images)

For all other SPOT camera lines, the Flatfield Correct option is used to correct for display problems associated with uneven intensity or coloration in illumination, or to correct for artifacts (e.g., dust) in the optical system. Flatfield can still be used for this purpose with SPOT Insight cameras. But, because SPOT Insight cameras use a color mosaic chip, Flatfield correction serves a new primary purpose. In the production process for color mosaic CCD chips, the RGB colors are applied directly to the pixels on the chip. This process often results in an uneven color density around the outer 30-40 rows of the CCD chip, causing a "halo" appearance in some image captures, particularly those with low contrast. The Flatfield option can be used to correct this problem.

Note: Flatfield correction with the SPOT camera refers only to the correction of uneven lighting. It should not be confused with the correction of optical field flatness.

Flatfield correction works by taking a picture of the brightfield background without the specimen in the field of view. This image lets the SPOT software know, on a pixel-by pixel basis, which areas in the field of view are receiving less illumination than the brightest areas. When taking a picture that contains a specimen, the software uses this information to accurately increase the brightness of each pixel in the dimly illuminated areas. The resulting image gives the appearance of an even illumination.

To use the **Flatfield** option, follow these steps:

1. Ensure that the specimen is under the desired objective and in focus.
2. Control-click on the **Flatfield** drop-down list and click on the **Add** option. The **Get Flatfield** dialog appears.
3. Remove the slide from the field of view.
4. Click on Begin. When the program finishes computing the exposure times and acquiring the flatfield file, the **Flatfield** dialog prompts you to name the file:
   a) Enter a name. Because flatfield image files are different for each objective, you might want to name the file according to the objective's magnification (e.g., 10x).
   b) Click on OK. If you do not specify a location, the program automatically saves the flatfield file to the **c:\SpotCam\Fltflds** directory.

Note: The default file location varies, depending on where you installed the SPOT program.

5. Move the specimen back into the field of view, and perform the image capture, using either the **Preview** or the **Acquire** option.

Color Enhancement (Captured Images)

The Color Enhancement option optimizes image captures by closely matching the final capture to the image as seen through the microscope. To optimize the final image, the SPOT program processes the raw image data to produce a final image that matches the view seen through the microscope.

If you want an image capture to match the live image view, you will want to leave Color Enhancement off. Color Enhancement applies only to captured images.

Fit to Window

In addition to automatically resizing images to fit within the live image window, the Fit to Window option also determines cursor behavior in the live image view.

- When the **Fit to Window** option is checked, the live image window **cursor** becomes a **zoom tool**. To zoom in on a portion of a live image, click and drag over the area that you want to zoom in on. To return to the non-zoomed image, click on the **Full Chip** option.
- When the **Fit to Window** option is not checked, the cursor is a cropping tool. If you select a portion of an image, the program displays only the selected area and discards the rest of the image. To return to the non-cropped image, click on the **Full Chip** option.

These points apply *only* when the **Always Show Full Chip in Live Mode** (see above) is *not selected.* For more details, refer back to the **Chip Imaging Area** section (see above).

More (Additional Orientation and Exposure Settings)

When you click on **More**, the program displays a dialog of additional orientation and exposure settings for the 3rd Party Interface, as shown at right.

Flip

Specify whether images will be flipped in the live image view and during the image capture process.

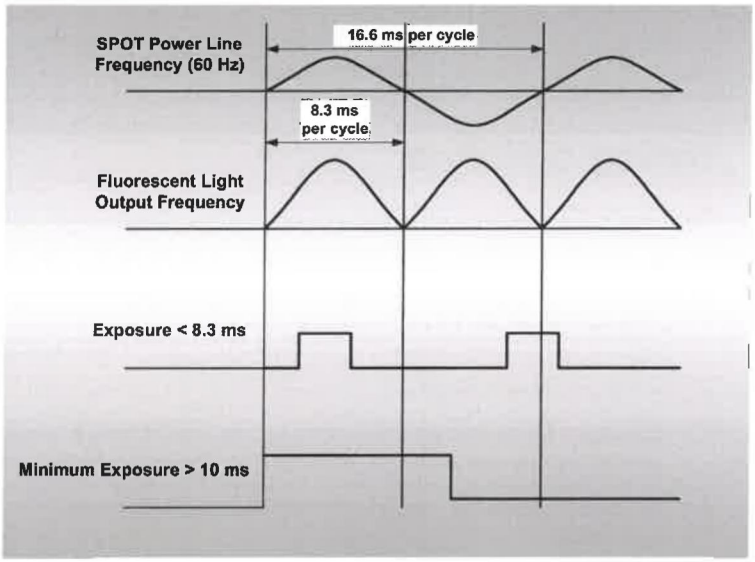
- Select **Horizontal** to flip the image from left to right.
- Select **Vertical** to flip the image from top to bottom.
- Select both options to flip the image from left to right and from top to bottom.

Rotate 90°

Specify whether images will be rotated in the live image view and during the image capture process.

- Select **None** if you do not want the image to be rotated.
- Select **Right** to rotate the image clockwise 90 degrees.
- Select **Left** to rotate the image counterclockwise 90 degrees.

Exposure Limits – Minimum



Setting a Minimum Exposure to Accommodate Varied Light Sources

Because SPOT Insight cameras are capable of extremely short exposure times, light sources with intensities that vary with the waveform of the 60 Hz line supply (e.g., fluorescent lamps, 120 volt incandescent bulbs, and 6 volt 20 watt halogen lamps) can cause the Live Image window to flicker and result in incorrect brightness and color balance readings in captured images. This option allows you to correct for these problems by limiting the camera to a minimum exposure time that is greater than the pulse interval of the light source. Consider the illustration to the right. In this case, the pulse interval of the fluorescent light source is 8.3 ms. Entering a Minimum Exposure of 10 ms ensures that the camera's exposure time is greater than the pulse interval of the light source. To enter a minimum exposure, check the **Minimum** box, and enter a value in milliseconds.

Exposure Limits – Live Image Maximum

Because The Live Image Maximum option helps you to avoid unnecessarily long exposures when viewing a live image. Consider the following situation: When you change from a lower resolution objective to a higher resolution objective, the camera detects a brief interval of blackness. When the Auto-Brightness option (see above) is enabled, it compensates by modifying the exposure time to maintain a constant brightness level. This results in you having to wait for the camera to compute a lengthy and incorrect exposure for the black interval. Using the **Live Image Maximum** option, you can limit this exposure time. For most cases, Diagnostic Instruments recommends a value of 0.5 seconds.

Similarly, the Live Image Maximum option can also be used in cases where the microscope's light path selector is incorrectly set.

Noise Filter

The Noise Filter corrects for the effects of electrical or thermal noise. When capturing dim images with lengthy exposure times, the noise filter acts as a "virtual cooler" for the Insight camera, eliminating "hot pixels."

The Noise Filter checks each pixel on the CCD chip as described below:

1. The program compares the value of each red pixel with the values of the eight neighboring red pixels.
2. If none of the eight neighboring red pixels has a value within ±15 % (i.e., the threshold value that the SPOT program uses) of the value for the red pixel being checked, the value for the red pixel being checked is replaced with the average value of the eight neighboring red pixels.

3. The program repeats this procedure for the values of the green and blue pixels being checked.

To activate the Noise Filter, check the Noise Filter box. The program runs the noise filter each time an image is captured using the **Preview** or **Acquire** options.

Taking a Picture

From the SPOT 3rd Party Interface, you can capture images in different ways:

- **Preview** - You can capture a **Preview** image. Preview images remain available in the 3rd Party window for basic editing and serve as the basis for the final image capture. When you click on the **Preview** button, the **Get Image** dialog displays the capture status as it is captured, and the captured image then replaces in the Live Image in the window. Note that the status bar indicated that you are viewing a Preview image.
- **Transfer** - If you like the Preview image, you can **Transfer** it image directly to the third party application that you are using. The Transfer button is active only for Preview image captures.
- **Acquire** - Capture an image and have it automatically transfer to (open in) the third party imaging application.

Appendix A: Electronic Imaging Theory and SPOT Cameras

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Introduction

This appendix is a brief overview of the operational theory behind SPOT digital cameras. The next two sections, Digital Photography and Color Images, provide a short introduction to electronic imaging theory; the following section describes how this theory applies to the SPOT cameras.

Digital Photography

Modern electronic imaging is based on the *charged coupled device (CCD)*. All of today's digital cameras have a CCD chip or chips. In digital cameras, the CCD chip/sensor replaces the film of traditional cameras as the means by which the camera records the image. The surface of the CCD chip is composed of light-sensitive cells arranged in a checkerboard pattern. Each cell of the checkerboard is known as a picture element, or more commonly, a *pixel*. The following is a simplified description of what happens when you take a picture with a digital camera:

1. The camera's optical system forms images on the "checkerboard" of pixels.
2. The CCD is exposed to the image for a period of time.

   During this period, each photo-sensitive cell receives photons of light, converts the photons to electrons, and then stores the electrons in the cell. The process by which each cell accumulates electrons can be compared to a well filling with water. As more light hits a cell, the electron level in the well rises. The more electrons that are in the cell, the more voltage it will have when read by the digital camera.
3. Following the exposure, a digital camera does three things:
   a. It measures the voltage of each cell.
   b. It converts the voltage to a binary number.
   c. It transmits this number down a cable to your computer.
4. The computer reconstructs the image by assigning a brightness value to each pixel in the final image. Each brightness value is proportional to the voltage of the corresponding cell on the CCD chip.

Color Images

Because a CCD chip is inherently monochromatic (black and white), color filters must be used to extract the color information from the image. There are three basic design methodologies.

- **Single CCD design using color masked pixels (SPOT Insight camera)**
  Digital cameras designed in this manner have one CCD chip with color filters physically bonded to each pixel on the chip. Some pixels get red filters, some pixels get green filters and some pixels get blue filters. Since each pixel is only able to measure the intensity of one color, the intensity of the missing two colors for this pixel must be estimated, based on nearby pixels that have precise measurements of the missing colors. For example, a pixel that has a green filter will have an exact measurement of the green color value, but the red and blue values must be estimated, based on the nearest red and blue pixels. This estimation is known as *interpolation*.

  Pros and Cons: A digital camera designed in this way is inexpensive and can freeze moving images with a single exposure. However, because they interpolate brightness values, such cameras cannot provide as high of a resolution as that provided from a non-filtered CCD chip.

- **Three CCD design using color beamsplitter**
  This design uses three CCD chips. The incoming image first goes through a color beam splitter which directs the red light to one chip, the green light to a second chip and the blue light to a third chip. The chips are very accurately aligned so as to achieve near perfect registration between the three chips. The red, green, and blue values for each point on an image are measured by the corresponding pixel on each of the three chips.

  Pros and Cons: This design produces an image that retains the high resolution of the individual chips. This type of camera can also freeze moving images with a single exposure. However, because most of the cost in a high resolution CCD camera is in the CCD chip itself, three chip cameras are far more costly than their single chip counterparts.

- **Single CCD design that takes three pictures (three pass method) (SPOT and SPOT RT cameras)**
  Cameras designed in this way expose a single CCD chip three times, once to red light, once to green light, and once to blue light. One variant in this type of camera is how the chip is exposed to the different colors of light. Some cameras switch individual red, green and blue glass filters in front of the chip for each exposure. Others use a liquid crystal filter that changes from red to green to blue as different voltages are applied to it. By exposing the chip three times, each cell on the CCD is able to measure all three color values.

  Pros and Cons: This technique attains the high resolution of the three CCD design without the high cost associated with using three chips. However, because these cameras require three exposures, they cannot freeze moving images.

SPOT Digital Cameras

In order to provide you with an understanding of what happens when you take a picture, the following sections highlight some of the basic operating principles of SPOT cameras.

Pixel Digitization

Following each exposure, SPOT reads the CCD chip and digitizes each pixel as it comes off of the CCD chip, and before it is sent through the cable into the computer. This technique minimizes noise and maintains the lack of distortion that is inherent with CCD chip geometry. The SPOT Insight cameras use two methods of pixel digitization, one for live imaging, and one for image capture:

- **Live imaging (RT and Insight)**
  To provide the fastest possible frame rate, SPOT RT and Insight cameras use two separate circuits to digitize the left and right halves of the CCD simultaneously.

  Pros and Cons: This method provides an extremely fast display when using SPOT's Live Image feature. The challenge comes in achieving a good brightness match between the two image halves. Our current algorithm achieves a good match with bright images. However, with dim images, mismatches can occur, resulting in a "split" appearance, where one half of the image is brighter that the other half. A "split" Live Image view is **not** indicative of what your image capture will look like.

- **Image capture (all SPOT models)**
  To provide the most accurate image captures, SPOT cameras use a single circuit to digitize the entire CCD chip.

  Pros and Cons: Because a single circuit is used to digitize the entire CCD chip, all pixels are inherently matched. However, this method provides a slower download than the two circuit method used for live imaging.

Gain

Normally an auto exposure routine in a digital camera sets the exposure time so that the brightest pixel on the CCD chip is completely "filled" with electrons, or is "full well" (see the Digital Photography section). This simple technique works well for bright images, but, as an image gets dimmer, exposure times can get uncomfortably long.

With the SPOT camera you can shorten exposure times by filling the brightest pixel on the CCD chip to less than full well, and then using this lower level as *full scale*. The inverse fraction of full well that the brightest pixel on the CCD is filled to is known as the *gain*. For example, filling a pixel to half of full well cuts the exposure time in half. Since the exposure time is cut in half, we call this a gain of 2.

The following table illustrates the relationship between gain, the fraction of full well that the brightest pixel is filled to, and the effect of gain on exposure time:

Gain	Fraction of Full Well	Exposure Time (sample)
1	1	80 seconds
2	½	40 seconds
4	¼	20 seconds
8	1/8	10 seconds
16	1/16	5 seconds

An 80 second exposure at a gain of one turns into a 5 second exposure at gain 16. This is good in terms of the exposure time. Unfortunately, background noise is directly proportional to gain. It is 16 times higher at a gain of 16 is than at a gain of one.

An Automated Exposure Process

Normally, for any given image, trial and error would have to be used to determine the optimal combinations of gain and exposure time. In order to eliminate the need for trial and error experimentation, the SPOT camera automatically determines this information by sampling light levels before each exposure. Based on the light levels and the user-determined Auto-Gain Limit, SPOT determines the optimal gain setting and exposure time for the image. For details on using the Auto-Gain Limit feature, refer to Ch. 5 – Preparing to Take a Picture.

Appendix B: Specifications

Camera and Filter
Insight Color – Color
Insight B/W – Monochrome

CCD Information
Insight Color – KAI 2000 Color Mosaic
Insight B/W – KAI 2000 Monochrome
Imaging Area – 11.8mm x 8.9mm
Active Pixels – 1600 x 1200 (1.92 million)
7.4 µm square pixels
Full well charge capacity - 31,500e

Insight Color Image Resolution
Live image – 800 x 600
Captured image – 1600 x1200

Insight B/W Image Resolution
Live image – 1600 x 1200
Captured image – 1600 x1200

Bit Depth – Insight Color
Sampled at 30 bit RGB
Saved file formats: 24 bit, 36 bit, or 48 bit (RGB)

Bit Depth – Insight B/W
Sampled at 10 bit monochrome
Saved file formats: 8 bit, 12 bit, or 16 bit (mono)

Pixel Readout Rate
Live images – 36 Mhz (2 channel x 18 Mhz)
Captured images – 18 Mhz (1 channel x 18 Mhz)

Live Image Frame Rate
733 PIII w/810e Graphics
Insight Color - 15 frames/s
Insight B/W – 15 frames/s

Acquisition Time to Monitor
(processor dependent)
Insight Color < 6 seconds
Insight B/W < 1 second

A/D Converter Full Scale Setting
31,500e (when gain = 1)

Signal to Noise Ratio
58 dB

Anti – Blooming Factor
300 times (nominal); 100 times (minimum)

Exposure
Automatic and User-Defined
Range – 1 ms – 536 seconds
(1 saturated hot pixel @ 5 sec. typical)

Lens Mount
"C" Mount
Flange to rear lens clearance - 0.46" (11.7 mm) max.

Computer Interface
PCI bus card (supplied)

External Shutter Control
BNC, TTL level output

Power Requirements
100 –240 VAC, 47 – 63 Hz

Weight
Camera - 1.5 lbs.
Power supply - 0.5 lbs.

Certifications
CE, FCC, Class A

Computer Requirements
PC - Pentium 166 or greater
Windows 95/98/00/NT/ME/XP

Mac – Power PC w/PCI bus
Mac OS 8.6 or greater

RAM – 64 Mb RAM required, 256 Mb recommended.

Video card – Support of 24 bit RGB at the desired monitor resolution.

Software Features
- Color live image window
- Image capture window
- Pre-defined image types
- Auto exposure
- Auto white balance
- Flatfield correction
- Zoom and pan window
- Image enhancement tools
- Annotation
- Calibration mark
- Measurement – length, perimeter area, angle
- Print layout dialog
- On-line help menu
- Image archiving database
- Report generator
- Sequential imaging

Image File Formats
TIFF, TIFF-JPEG, JPEG, BMP, PICT

File Size

Insight Color	Insight B/W
24 bit - 5.49 MB	8 bit - 1.83 MB
36 bit - 8.24 MB	12 bit - 2.75 MB
48 bit – 10.99 MB	16 bit - 3.66 MB

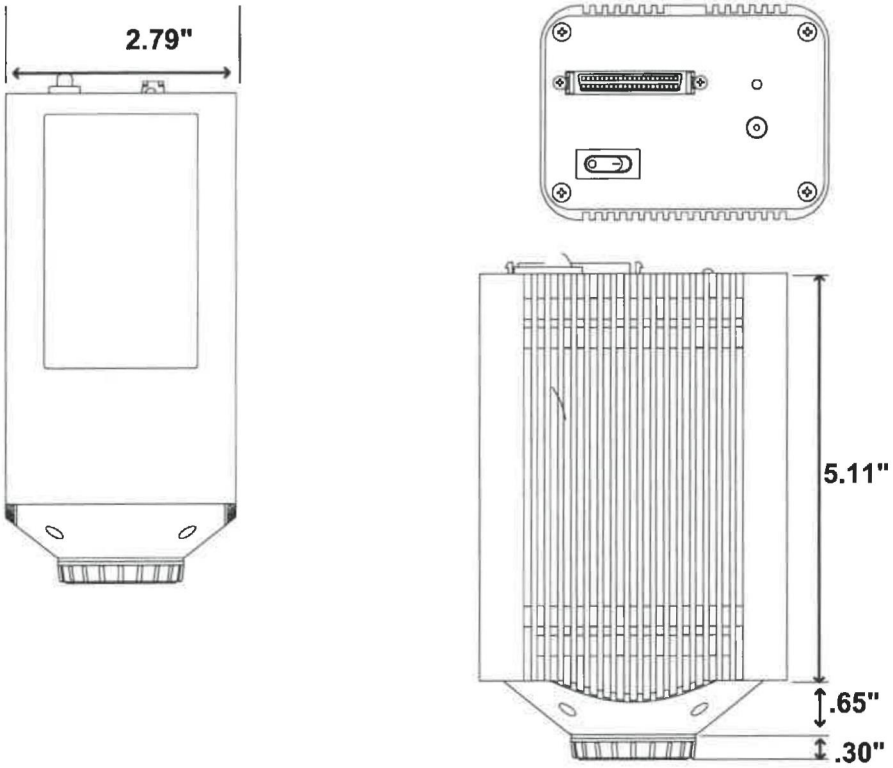
Drivers
TWAIN for Windows 95/98/NT/2000/ME;
AppleEvent for Mac OS

Third Party Software
See Appendix G.

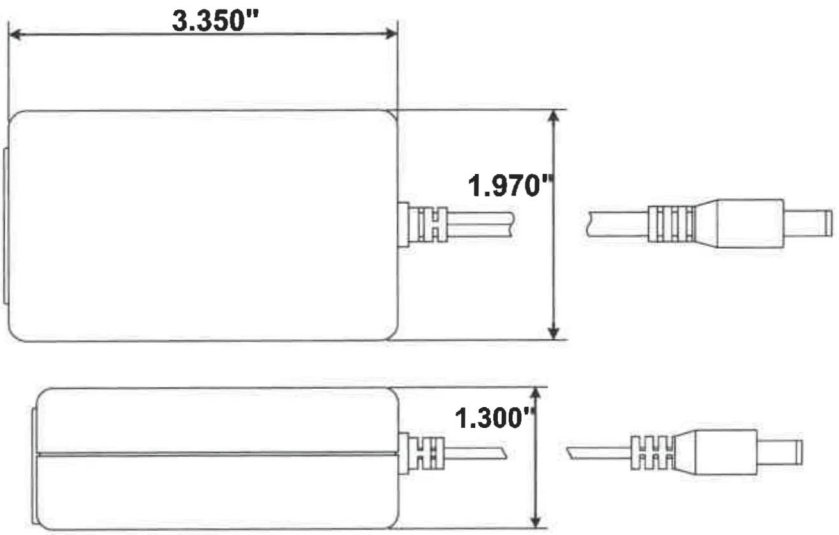
Couplers
D10XXC – 1x C-Mount Coupler (Diagnostic Instruments)
Alternatives made by Diagnostic Instruments:
- **HRU060-CMT coupler (0.60x)** and the appropriate bottom clamp.
- **HRD100-CMT coupler (1x)** and the appropriate bottom clamp.

Lens
C- Mount lens for 1" format CCD

Camera Dimensions



Power Supply Dimensions



Insight Series Catalog Information

There are two cameras in the SPOT Insight series of cameras: Insight Color and B/W (monochrome). The following table shows each camera with the standard and special order options:

Catalog Part Numbers	Model Number	Description	Stock/Special Order
IN320	3.2.0	SPOT Insight Color "C" mount camera *with IR filter*	Stock
IN310	3.1.0	SPOT Insight B/W (monochrome) "C" mount camera *with IR filter*	Stock
IN305	3.0.5	SPOT Insight B/W (monochrome) "C" mount camera *without IR filter*	Special Order

FCC "Class A" Product Warning

All SPOT cameras fall into the Class A category of the FCC (Federal Communications Commission) emission standard EN55022/CISPR22. The following is the FCC's standard warning for this type of device:

WARNING:
This is a Class A Product. In a domestic environment this product may cause radio interference in which case the user may be required to take adequate measures.

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Appendix C: Keyboard and Toolbar Button Commands

Command	Keystroke or F-key	Toolbar Button	Basic and/or Adv. Mode	Windows and/or Mac
File Menu				
Open Image File	[F3]		Both	Both
Show Image File Thumbnails	[F5]		Both	Both
Run Report	[F6]		Advanced	Windows
Reload			Advanced	Windows
Save	[F2]		Both	Both
Save As	[Ctrl] + [A]		Both	Windows
Save to Database	[Ctrl] + [D]		Advanced	Windows
Save All	[Ctrl] + [F3]		Both	Windows
Close	[Ctrl] + [F4] ⌘ + W		Both	Both
Print	[Ctrl] + [P] ⌘ + P		Both	Both
Exit Quit	[Alt] + [F4] ⌘ + Q		Both	Both
Database menu				
Search			Advanced	Windows
Edit menu				
Undo	[Alt] + Bksp ⌘ + Z		Both	Both
Copy to Clipboard	[Ctrl] + [C] ⌘ + C		Advanced	Both
Add Measurement	[Alt] + [M] ⌘ + M		Advanced	Both

(cont.)

Command	Keystroke or F-key	Toolbar Button	Basic and/or Adv. Mode	Windows and/or Mac
Sequence menu				
Play Forward	[Ctrl] + [Space] ⌘ + [Space]		Advanced	Both
Play Reverse	[Ctrl] + [Bksp] ⌘ + [Bksp]		Advanced	Both
Stop Play/Resume Play	[Enter]		Advanced	Both
Step Forward	[Space]		Advanced	Both
Step Back	[Backspace]		Advanced	Both
View menu				
Fit to Window	[Ctrl] + [F] ⌘ + F		Both	Both
Zoom	[Ctrl] + [Z]		Both	Both (Macs – button only)
Show Gridlines	[Ctrl] + [G] ⌘ + M		Both	Both
Show Pan Window	[Ctrl] + [W]		Both	Both (Macs – button only)
Image Date/Tme	[Ctrl] + [T] ⌘ + T		Both	Both
Full Screen	[F 7]		Both	Both
Show Image Settings			Basic	Both
Camera menu				
Live Image	[F11]		Both	Both
Get Image (exposure from setup)	[F9]		Both	Both
Get Image (last used exposure)	[F8]		Advanced	Both
Compute Exposure	[F10]		Advanced	Windows
Compute White Balance Values	[Ctrl] + [F10]		Both	Both
Non-menu driven commands				
Access the Image Setup window	[F12] [Ctrl] + [F 12] (OSX 10.1 and higher)		Advanced	Both

Appendix D: Selected Error Messages

In the following table, unless indicated, the error messages apply to all SPOT camera models and to both Windows and Mac operating systems.

Error Message	Cause(s) and Solution(s)
A file could not be found (Mac)	Something went wrong in a file operation, usually due to a disk error. *Reboot the computer.*
At Least One Exposure time must be specified	You defined your image setup as "User-Defined" (see Ch. 5), but did not enter exposure times. *Enter the exposure times or use the Compute Exposure option to have the camera compute exposure values for you. If you have captured an image previously, you can use the Recall Previous Exposure option.*
Camera Error	There is damage to the camera head or the shutter is malfunctioning. *Contact Diagnostic Instruments.*
Error deleting file (Mac)	Something went wrong in a file operation, usually due to a disk error. *Reboot the computer.*
Error modifying registry	Same as above
Error reading camera information (RT cameras only)	There is damage to the camera head, problems with the interface card, or a bad connection cable *Contact Diagnostic Instruments.*
Error reading file (Mac)	Something went wrong in a file operation, usually due to a disk error. *Reboot the computer.*
Error writing file (Mac)	Something went wrong in a file operation, usually due to a disk error. *Reboot the computer.*
Exposure time too short for camera	The brightness of the image requires an exposure time that is too short for the camera. *Add a neutral density filter to dim the image without changing the color balance.*
Flatfield file is incompatible with image setup	The pixel bit depth (bpp) of your image setup does not match the pixel bit depth of the flatfield file (e.g., the image setup is 8 bpp and the flatfield file is 24 bpp). *Modify either the image setup or the flatfield file so that the two match.*
Images have different bit depths	The images that you are trying to merge, add, or subtract have different pixel bit depths. *Change one of the images so that their pixel bit depths are the same.*
Images must have the same dimensions	The images that you are trying to merge, add, or subtract are two different sizes. This may be because you cropped or binned an image for viewing purposes. *Ensure that the two images have the same dimensions. You might simply use un-cropped/non-binned versions of the images.*

Image too dim	There is not enough light to capture the image. This error message appears for various reasons, so you might have to experiment to increase the light. Possible solutions include: • *Adjusting the microscope settings to let in more light* • *Binning the image (see Ch. 5) to increase camera sensitivity* • *For RT Slider users, sliding the color filter to the B/W position*
JPEG Quality value must be between 1 and 100.	When saving an image, or defining database (Ch. 9) or sequential image (Ch. 6) preferences, you entered a value less than one or greater than 100 (for a JPEG or TIFF JPEG format image). *Re-enter a value between one (highest compression, lowest quality) and 100 (lowest compression, highest quality).*
Memory is getting full. Please try to alleviate the problem by closing some documents. (Mac)	The system is running low on memory. *Increase the memory allocation for the SPOT program.*
No response from the camera. Make sure the camera is connected and the power is on ***(when you first try to open the SPOT program)***	This message appears for various reasons. The most typical are: • The power supply is not turned on. • The PCI board is not seated properly. • A cable is loose. *Turn on the power supply, ensure that the PCI card is properly seated, or ensure that the cables are firmly connected to the camera/computer/power supply. Refer to the* Installation Guidelines *section of the manual for details on installing the PCI card and connecting the camera to the microscope and computer.*
Not a supported bit depth image	You tried to open an image with a pixel bit depth that is not supported by the SPOT software. *Using another imaging program change the pixel bit depth of the image to one of the four depths that the SPOT program supports: 8, 12, 16, 24, 36, or 48. Note that while 16 and 48 bit per pixel images can be opened by the SPOT program, they are treated as 12 bit per channel images.*
Out of Memory (Mac)	The system is running low on memory. *Increase the memory allocation for the SPOT program.*
Place the color filter in the 'Color' position **Place the color filter in the 'B/W' position** ***(RT Slider only)***	These messages appear when you are trying to capture an image and the position of the sliding color filter does not match the image setup filter definitions. For example, you will see this message if you selected the Use Color Filter option for the image setup, but the sliding filter is out (in the B/W position). Note that you will see this message only if the **Monitor Camera Color Filter Preferences** option is selected on the Preferences window.The camera will not allow you to capture an image without correcting this discrepancy. *Either move the filter to the correct position, or change the image setup definition to match the position of the color filter. Alternatively, you can deselect the Monitor Camera Color Filter Preferences `option and the program will not prompt you with error messages.*

Printer Error	The printer software is not installed. In order for the SPOT program to work correctly, you must have a print driver installed on your system. The printer does not necessarily need to be attached to the computer, but the drivers need to be installed. *Install a printer driver – follow the manufacturer's instructions for installing the printer that you plan to use.*
Software is incompatible with camera hardware	The causes of and solutions to this error message, which appears when you try to run the SPOT program for the first time, vary according to the camera model: • For both RT and non-RT cameras, this message appears if, during the installation process, you specified the wrong interface card for the camera. *Reinstall the SPOT software and specify the correct interface card. Refer to the* Installing the Software *section of the Installation Guidelines chapter for more details.* • For non-RT cameras, this error message indicates that the chipinfo.dat file is from an earlier version of the SPOT program. *Contact Diagnostic Instruments and request an updated chipinfo.dat file.*
Spot cannot be launched in basic mode due to a disk or permissions error (Mac)	The basic mode launcher failed to write a necessary file in the preferences folder. *Reboot the computer. If the problem persists, run Disk First Aid, located in the Utilities folder of the boot drive.*
Spot cannot be launched, because the application cannot be located. (Mac)	The SPOT Basic application must be in the same folder as the copy of SPOT the user is trying to start. *Move the Basic mode application file (SPOT 3_4) into the same folder as the Advanced mode application file.*
Spot cannot run in the Classic environment under OS X. (Mac)	This message appears if you try to run the OS 9 version of the SPOT software (either the full application or the 3rd Party Interface) in the Mac OS X environment. SPOT closes immediately following the display of this error message. *Re-open the SPOT software in Mac OS 9.*
The Spot Prefs folder is missing **(Mac)**	The folder, which is necessary in order to run the Basic mode program (among other things) is missing. *Run the SPOT application (i.e., double-click on it) to create the folder.*
This version of Spot only runs on OS X. (Mac)	This message appears when you try to open the Carbon (OS X) version of the SPOT software (either the full application or the 3rd Party Interface) in Mac OS 9. SPOT closes immediately following the display of this error message. *Re-open the SPOT software in Mac OS X.*

Video driver is not set to true color mode (millions of colors). Displayed images may not appear with the correct colors.

The video driver for your graphics card is not set to True Color mode.

From the Windows Control Panel, change the graphics card settings to True Color (24 bit) or True Color (32 bit): Select
Start>Settings>Control Panel>Display>Settings>
Color Palette **(Win 95/NT) or** Colors **(Win 98)**
and choose True Color **(Win NT only)**, True Color (24 bit) **or** True Color (32 bit) ***from the drop-down list.***

Appendix E: Converting Previous Version SPOT Databases

If you have SPOT databases that were created with an earlier version of the SPOT software (2.1 or earlier), these databases must be converted for use with the current version of the software (3.5). Follow these steps:

1. Access the files on the software CD that came with your camera. In addition to the documentation and program folders, you should see a Utilities folder.

2. Open the Utilities folder.

3. Double click on the **convdb.exe** icon. The program automatically converts your databases.

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Appendix F: Third Party Software

The following table lists major third party imaging software systems and their compatibility levels with the SPOT, SPOT RT, and SPOT Insight cameras.

			Drivers are offered by the 3rd Party software manufacturer			*Drivers are included with the SPOT software*	
Company	**Software**	**System**	**SPOT (1401E & 0401E)**	**SPOT RT Driver**	**SPOT Insight Driver**	**TWAIN (Windows)**	**3rd Party Interface (Mac)**
Adobe	Photoshop	PC/Mac	NO	NO	NO	YES	YES
Cerner	CoPathPlus	PC	NO	NO	NO	YES	NO
Clemex	Vision	PC	NO	YES	YES	YES	NO
Compix	C-Imaging	PC	YES	YES	NO	YES	N/A
Delta Sistemi	Biomed	PC	YES	YES	YES	YES	NO
Empix	Northern Eclipse	PC	YES	NO	NO	YES	N/A
IATIA	QPM	PC	NO	YES	YES	NO	NO
Improvision	Open Lab	Mac	YES	YES	YES	N/A	YES
Intelligent Imaging Innovations	3i	PC	YES	YES	NO	YES	NO
Media Cybernetics	Image-Pro (4.x or higher)	PC	YES	YES	YES	YES	N/A
Midwest Info. Systems	Paxit	PC	NO	NO	PENDING	YES	NO
NIH - Public Domain	NIH Image	PC/Mac	NO	NO	NO	NO	YES
QED	Camera Plugin	PC/Mac	NO	YES	YES	NO	YES
Scanalytics	IP Labs	PC/Mac	YES	YES	YES	YES	YES
Soft Imaging System	AnalySIS	PC	NO	YES	YES	NO	NO
Universal Imaging	Metamorph	PC	YES	YES	YES	YES	NO

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